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Molecular Complexes of Flavins. The Crystal Structure of Lumiflavin–Bis(naphthalene-2,3-diol) Trihydrate

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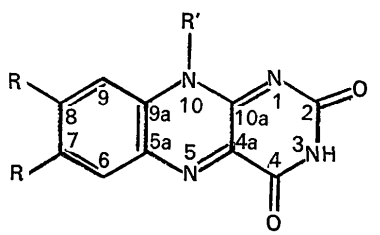
Crystals of the reddish-orange molecular complex lumiflavin–bis(napthalene-2,3-diol) trihydrate are triclinic, $P\bar{1}$, with $a=9{\cdot}185$ (9), $b=10{\cdot}68$ (1), $c=17{\cdot}46$ (1) Å, $\alpha=116{\cdot}27$ (2), $\beta=85{\cdot}05$ (2), $\gamma=101{\cdot}20$ (2)°, $Z=2$, and $\varrho_{calc}=1{\cdot}389$ (3) g cm^{-3}. The structure has been refined by least-squares calculations to $R=0{\cdot}081$, with 1448 reflections measured by scintillation counter used as data. One naphthalenediol forms an alternating donor–acceptor stack with the lumiflavin; the second fills gaps between stacks. The donor on one side of the flavin overlaps the benzopyrazine region with an oxygen making the closest approach of 3·11 Å to the mean flavin plane. On the other side, overlap involves the pyrazine and pyrimidine rings, with a closest approach of 3·26 Å. Both naphthalenediol molecules are internally hydrogen bonded; the flavin molecules form base pairs using N[3] and O[4]. Flavin atoms N[1], O[2], O[4], and N[5] form additional hydrogen bonds to naphthalenediol or water.

Introduction

Flavins, or 7,8,10-substituted isoalloxazines (I), are widely occurring cofactors in biological oxidation–reduction reactions.



(I)

The suggestion has been made (Szent-Gyorgyi, 1960) that the initial step in some such reactions may be formation of a transient charge-transfer complex. A stable charge-transfer complex can, in fact, be formed by the flavoprotein lipoyl dehydrogenase with nicotinamide adenine dinucleotide, NAD (Massey & Palmer, 1962). Furthermore, flavin π complexation with aromatic enzyme constituents is likely to be an important factor in flavin–apoprotein bonding, inasmuch as most flavin cofactors are not covalently bound. An understanding of the functioning of this widespread and important class of enzymes must thus rest in large part on the analysis of the intrinsic π-bonding capabilities of the aromatic flavin nucleus.

To clarify the nature of π complexation and also to identify the major hydrogen-bonding sites in neutral flavins, several crystals have been prepared in this laboratory (Wells, Trus, Johnston, Marsh & Fritchie, 1974; Kuo, Dunn & Fritchie, 1974) which use the common donor naphthalene-2,3-diol and various isoalloxazine species which should be sterically and electronically fairly similar to *bona fide* flavin cofactors, at least in the vicinity of the aromatic redox-active moiety. This donor was chosen from among a number of aromatic compounds studied because it most consistently gave crystalline complexes, because similar complexes have been studied optically (Tollin, 1968, and references therein), and because similar compounds, notably tyrosine and ubiquinone, are among the possible species which may interact with flavins *in vivo*. Tyrosine has recently been shown to be one of two aromatic residues aiding in binding riboflavin 5′-phosphate (FMN) to *Desulfovibrio vulgaris* flavodoxin (Watenpaugh, Sieker & Jensen, 1973).

Experimental data

Reddish-orange crystals of the complex lumiflavin bis(naphthalene-2,3-diol) trihydrate, $C_{13}H_{12}N_4O_2.2C_{10}H_8O_2.3H_2O$, were grown by slow evaporation of 1:1 acetone–water solutions of the components. A large excess of the naphthalenediol was used to increase the solubility of lumiflavin.

Preliminary Weissenberg photographs showed C_i Laue symmetry; space group $P\bar{1}$ has been confirmed by the structure analysis. Lattice constants, refined by least-squares fitting of $\sin^2\theta$ values for a number of reflections which were centered manually on a Picker four-circle card-controlled diffractometer, are $a=9{\cdot}185$ (9), $b=10{\cdot}68$ (1), $c=17{\cdot}46$ (1) Å, $\alpha=116{\cdot}27$ (2), $\beta=85{\cdot}05$ (2), and $\gamma=101{\cdot}20$ (2)°.* Mo $K\alpha$ radiation was used for these measurements. With two formula groups per unit cell, the calculated density is 1·389 (3) g cm^{-3}.

* The Delaunay cell has constants $a=9{\cdot}185$, $b=12{\cdot}66$, $c=15{\cdot}93$ Å, $\alpha=97{\cdot}50$, $\beta=92{\cdot}04$, and $\gamma=124{\cdot}17$°. The transformation matrix from the cell actually used to the Delaunay cell is

$$\begin{pmatrix} -1 & 0 & 0 \\ 1 & 1 & 0 \\ 0 & -1 & -1 \end{pmatrix}.$$

All independent intensities to a sin θ/λ limit of 0·5 Å^{-1} were measured on the same Picker diffractometer by a θ–2θ scan at a rate of 1° min^{-1}, with a 2° scan range. Mo $K\alpha$ radiation, filtered through Zr foil, was used for the measurements, with a Na(Tl)I scintillation detector serving as a counter. A pulse-height discriminator rejected the outer 10% of the Mo $K\alpha$ pulse distribution. Backgrounds were determined by stationary counts at either end of the scans. The net intensities, I, and their standard deviations, σ_I, were given by $I=[C-(t_C/2t_B)(B_1+B_2)]$ and $\sigma_I=[C+(t_C/2t_B)^2(B_1+B_2)]^{1/2}$, where C = scan count, t_C = time of count (120 s), t_B = time of each background (20 s), and B_1 and B_2 are the two background counts. Structure magnitudes and their standard deviations were derived from I and σ_I by use of the Lorentz and polarization factors. Each reflection with $I \leq 2\sigma_I$ was considered unobserved and was discarded. Of the roughly 3150 reflections measured, 1448 remained as data.

Structure solution and refinement

The central anthracene-like core of the lumiflavin and most of the naphthalenediol molecule which is nearly parallel to it (molecule *A*) were located by identification of the multiply superimposed vectors arising from these nearly centrosymmetric and similarly oriented molecular fragments (Patterson, 1939). A series of Fourier maps constructed by using these atoms for sign determination led to identification of the three water molecules and the remaining naphthalenediol. Least-squares refinement, using Hughes's linearized equations (Hughes, 1941) improved the heavy-atom positions and ellipsoidal thermal parameters. All hydrogen atoms were located in difference maps, except for one of the O(44) water hydrogen atoms and both of those on O(46). Hydrogen atoms bonded to carbon were periodically idealized in position, with the aid of difference maps calculated in the appropriate H_3 planes in the case of the methyl groups. The other hydrogen atoms were not moved.

The final refinement, using $1/\sigma_F^2$ as weight, where σ_F^2 is the larger of that derived from σ_I as described above, or $(0{\cdot}025\ |F_o|)^2$, converged at $R=0{\cdot}081$ and $R_w=0{\cdot}084$. In the last cycle, average and maximum shifts were approximately $0{\cdot}16\sigma$ and $0{\cdot}84\sigma$ for positional parameters, and $0{\cdot}16\sigma$ and $0{\cdot}38\sigma$ for thermal parameters. Tables 1 and 2 list the resulting positional and ellipsoidal thermal parameters; Fig. 1 gives the atomic numbering scheme. The isotropic thermal parameters used for the hydrogen atoms were 3·0 Å^2 for H(47) and H(48), 3·8 Å^2 for H(49)–H(60), 4·0 Å^2 for H(61), and 6·0 Å^2 for H(62)–H(77).* Major programs used were *LOKI*, a local crystallographic system, *GSET*4 (Prewitt, 1964), and *ORTEP* (Johnson, 1965). Atomic scattering factors were obtained from *International Tables for X-ray Crystallography* (1968) for all atoms except hydrogen (Stewart, Davidson & Simpson, 1965).

Table 1. *Positional parameters*

Heavy-atom parameters are $\times 10^4$, hydrogen-atom parameters $\times 10^3$. Figures in parentheses are estimated standard deviations.

	x	*y*	*z*
N(1)	2038 (9)	1350 (7)	4061 (4)
C(2)	1128 (12)	2163 (9)	4660 (6)
N(3)	911 (9)	3458 (7)	4706 (4)
C(4)	1622 (11)	4036 (9)	4183 (6)
N(5)	3373 (8)	3664 (8)	3015 (4)
C(6)	5043 (12)	3260 (9)	1809 (5)
C(7)	5968 (11)	2498 (9)	1211 (6)
C(8)	6128 (12)	1164 (10)	1161 (6)
C(9)	5356 (11)	673 (9)	1720 (6)
N(10)	3604 (9)	1049 (7)	2924 (4)
C(11)	2766 (12)	1869 (9)	3550 (5)
C(12)	2641 (10)	3170 (9)	3537 (5)
C(13)	4264 (11)	2836 (9)	2411 (5)
C(14)	4415 (11)	1507 (9)	2338 (5)
C(15)	460 (8)	1800 (6)	5196 (4)
O(16)	1431 (7)	5166 (6)	4226 (4)
C(17)	3681 (13)	−392 (9)	2835 (6)
C(18)	6824 (13)	3009 (10)	586 (6)
C(19)	7098 (12)	248 (11)	494 (6)
C(20)	7032 (12)	3240 (10)	3783 (6)
C(21)	7096 (12)	2006 (10)	3866 (6)
C(22)	7988 (12)	1099 (9)	3349 (5)
C(23)	8969 (11)	1407 (10)	2735 (6)
C(24)	9915 (12)	506 (11)	2176 (7)
C(25)	10778 (14)	802 (11)	1590 (7)
C(26)	10765 (12)	2066 (11)	1530 (6)
C(27)	9875 (13)	2977 (10)	2073 (6)
C(28)	8917 (11)	2676 (10)	2683 (6)
C(29)	7932 (12)	3584 (9)	3224 (6)
O(30)	6020 (8)	4032 (7)	4310 (4)
O(31)	6173 (8)	1684 (7)	4435 (4)
C(32)	−780 (11)	3450 (10)	8184 (6)
C(33)	98 (12)	3134 (10)	7426 (6)
C(34)	1527 (12)	2980 (9)	7446 (6)
C(35)	2183 (12)	3082 (9)	8167 (6)
C(36)	3688 (12)	3027 (9)	8233 (6)
C(37)	4279 (12)	3197 (11)	8949 (7)
C(38)	3368 (13)	3448 (11)	9691 (7)
C(39)	1908 (13)	3502 (10)	9664 (6)
C(40)	1274 (13)	3341 (9)	8903 (6)
C(41)	−215 (13)	3501 (11)	8897 (6)
O(42)	−2187 (8)	3662 (7)	8182 (4)
O(43)	−589 (9)	3036 (8)	6745 (4)
O(44)	4169 (8)	3467 (7)	5695 (4)
O(45)	6398 (8)	3380 (7)	6708 (4)
O(46)	7363 (10)	309 (8)	5056 (5)
H(47)	487	414	179
H(48)	534	−29	167
H(49)	790	20	340
H(50)	993	−37	225
H(51)	1140	13	116
H(52)	1147	234	111
H(53)	991	387	202
H(54)	793	449	320
H(55)	201	273	684
H(56)	427	277	765
H(57)	540	324	899
H(58)	371	366	1031
H(59)	134	362	1024
H(60)	−81	327	946

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30685 (9 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 1 (*cont.*)

H(61)	−50	400	550
H(62)	357	−110	218
H(63)	301	−65	323
H(64)	471	−39	305
H(65)	633	365	48
H(66)	705	227	0
H(67)	781	352	87
H(68)	813	82	50
H(69)	675	−19	−14
H(70)	728	−48	68
H(71)	730	330	630
H(72)	680	460	710
H(73)	470	380	630
H(74)	607	509	431
H(75)	543	243	486
H(76)	−274	334	758
H(77)	12	279	616

Description of the structure

Molecular geometry

Interatomic distances and angles not involving the hydrogen atoms are compared with literature values in Tables 3 and 4. Because of the apparently significant non-planarity of the naphthalene-2,3-diol not involved in complexing (see below), it is important to have an independent estimate of the errors in atomic positions. The root-mean-square difference of a lumiflavin bond length from the corresponding ideal value quoted by Wang & Fritchie (1973) is 0·015 Å, whereas the value estimated from the least-squares equations is about 0·016 Å. Similar r.m.s. differences for the two naphthalenediol molecules in comparison with the aver-

Table 2. *Anisotropic thermal parameters* ($\times 10^4$)

The thermal expression is $\exp[-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}l^2-\beta_{12}hk-\beta_{13}hl-\beta_{23}kl]$. Figures in parentheses are estimated standard deviations.

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	120 (14)	82 (9)	32 (3)	51 (18)	67 (12)	44 (8)
C(2)	145 (19)	71 (11)	28 (4)	−10 (24)	−26 (15)	27 (10)
N(3)	119 (14)	86 (8)	36 (3)	2 (18)	17 (12)	79 (7)
C(4)	115 (17)	67 (11)	53 (5)	40 (22)	−19 (12)	59 (10)
N(5)	96 (13)	119 (9)	18 (3)	67 (18)	17 (11)	54 (7)
C(6)	125 (13)	103 (11)	33 (4)	36 (22)	−8 (14)	74 (9)
C(7)	65 (15)	103 (11)	37 (4)	10 (22)	11 (14)	67 (9)
C(8)	107 (18)	135 (14)	47 (5)	22 (25)	11 (17)	72 (12)
C(9)	97 (16)	52 (11)	46 (5)	18 (21)	−8 (15)	29 (11)
N(10)	142 (15)	74 (9)	19 (3)	35 (19)	11 (12)	6 (8)
C(11)	143 (18)	87 (12)	13 (4)	62 (23)	11 (14)	3 (10)
C(12)	88 (15)	121 (10)	30 (4)	101 (17)	5 (13)	82 (8)
C(13)	115 (16)	94 (11)	33 (4)	80 (21)	17 (14)	57 (9)
C(14)	68 (15)	108 (12)	21 (4)	23 (22)	32 (14)	27 (10)
O(15)	186 (13)	133 (8)	44 (3)	101 (17)	57 (11)	100 (7)
O(16)	153 (11)	85 (7)	40 (3)	141 (13)	46 (10)	61 (6)
C(17)	207 (20)	85 (12)	42 (4)	114 (24)	68 (17)	72 (10)
C(18)	162 (20)	168 (13)	39 (4)	92 (25)	48 (16)	119 (10)
C(19)	143 (18)	165 (15)	29 (5)	143 (26)	49 (16)	40 (12)
C(20)	108 (18)	90 (12)	45 (5)	−22 (24)	−21 (16)	55 (11)
C(21)	141 (19)	111 (12)	39 (4)	50 (24)	−17 (16)	62 (10)
C(22)	161 (19)	72 (11)	23 (4)	−17 (24)	−24 (15)	31 (9)
C(23)	77 (15)	135 (12)	34 (4)	108 (21)	6 (14)	53 (10)
C(24)	121 (18)	136 (14)	52 (5)	58 (25)	17 (17)	72 (12)
C(25)	177 (21)	133 (14)	47 (5)	58 (27)	37 (18)	66 (12)
C(26)	125 (19)	155 (13)	37 (5)	0 (28)	1 (17)	69 (12)
C(27)	188 (21)	96 (12)	46 (4)	−6 (27)	−27 (17)	82 (10)
C(28)	99 (16)	138 (11)	37 (4)	62 (22)	−4 (14)	90 (9)
C(29)	124 (18)	62 (11)	42 (5)	−13 (23)	−32 (16)	32 (11)
O(30)	152 (13)	141 (8)	46 (3)	97 (12)	26 (11)	72 (7)
O(31)	145 (13)	158 (9)	60 (3)	115 (17)	61 (11)	105 (8)
C(32)	81 (16)	94 (12)	40 (5)	31 (23)	27 (15)	34 (11)
C(33)	152 (17)	126 (13)	38 (5)	113 (25)	49 (17)	51 (12)
C(34)	177 (20)	75 (12)	23 (4)	70 (25)	46 (12)	23 (10)
C(35)	142 (13)	120 (11)	36 (4)	97 (23)	60 (15)	85 (9)
C(36)	168 (20)	109 (11)	62 (5)	112 (24)	42 (17)	110 (10)
C(37)	89 (17)	139 (13)	82 (6)	76 (23)	22 (18)	115 (12)
C(38)	121 (19)	138 (15)	46 (5)	−48 (28)	−54 (13)	40 (13)
C(39)	173 (21)	96 (12)	47 (5)	−23 (26)	−10 (13)	65 (11)
C(40)	168 (19)	66 (11)	42 (4)	21 (24)	9 (16)	53 (10)
C(41)	149 (19)	137 (13)	28 (4)	15 (26)	18 (16)	63 (10)
O(42)	109 (12)	160 (9)	39 (3)	80 (16)	10 (10)	54 (8)
O(43)	210 (14)	247 (11)	34 (3)	206 (18)	21 (12)	7 (8)
O(44)	139 (12)	120 (9)	56 (3)	42 (10)	−30 (11)	55 (8)
O(45)	164 (13)	183 (9)	61 (3)	75 (18)	34 (12)	131 (8)
O(46)	228 (16)	462 (12)	150 (4)	295 (22)	109 (14)	443 (9)

ages of four other such molecules (Wells *et al.*, 1974; Kuo, Dunn & Fritchie, 1974) are 0·015 Å for the π-complexed naphthalenediol, molecule *A*, and 0·020 Å for the other naphthalenediol, molecule *B*. The r.m.s. average difference for all 48 bonds is 0·017 Å. It is thus likely that the standard deviations quoted are essentially correct, or at most are slightly underestimated.

Within the somewhat large standard deviations, all the molecules appear normal with respect to both bond lengths and bond angles. Even the unusual contraction of the angles C(21)–C(20)–O(30) and C(32)–C(33)–

Table 3. *Interatomic distances* (Å)

Lumiflavin

	This structure	'Ideal'*		This structure	'Ideal'*
N(1)—C(2)	1·37	1·368	C(6)—C(7)	1·36	1·370
C(2)—N(3)	1·40	1·407	C(7)—C(8)	1·42	1·413
C(2)—O(15)	1·24	1·212	C(7)—C(18)	1·52	1·501
N(3)—C(4)	1·37	1·361	C(8)—C(19)	1·50	1·503
C(4)—C(12)	1·49	1·484	C(8)—C(9)	1·38	1·380
C(4)—O(16)	1·22	1·216	C(9)—C(14)	1·41	1·395
C(11)–C(12)	1·43	1·452	C(14)–N(10)	1·42	1·388
C(12)–N(5)	1·32	1·298	N(10)–C(11)	1·35	1·362
N(5)—C(13)	1·37	1·371	N(10)–C(17)	1·49	1·476
C(13)–C(14)	1·40	1·411	C(11)–N(1)	1·32	1·311
C(13)–C(6)	1·41	1·409			

Naphthalenediol

	This structure	Literature average†		This structure	Literature average†
C(20)–C(21)	1·40	1·42	C(24)–C(25)	1·35	1·36
C(32)–C(33)	1·43		C(26)–C(27)	1·37	
C(21)–C(22)	1·35	1·34	C(36)–C(37)	1·33	
C(20)–C(29)	1·35		C(38)–C(39)	1·36	
C(33)–C(34)	1·36		C(25)–C(26)	1·40	1·38
C(32)–C(41)	1·37		C(37)–C(38)	1·43	
C(22)–C(23)	1·45	1·40	C(23)–C(28)	1·41	1·41
C(28)–C(29)	1·43		C(35)–C(40)	1·42	
C(34)–C(35)	1·39		C(20)–O(30)	1·38	1·36
C(40)–C(41)	1·41		C(21)–O(31)	1·37	
C(23)–C(24)	1·41	1·41	C(32)–O(42)	1·35	
C(27)–C(28)	1·43		C(33)–O(43)	1·36	
C(35)–C(36)	1·41				
C(39)–C(40)	1·42				

* Wang & Fritchie (1973).
† Average over four molecules from Wells *et al.* (1974), and from Kuo, Dunn & Fritchie (1974), assuming D_{2h} symmetry.

Table 4. *Bond angles* (°)

Lumiflavin

	This structure	Literature*		This structure	Literature*
N(1)—C(2)—N(3)	122	120·2 (3)	C(6)—C(7)—C(18)	122	120·8 (3)†
N(1)—C(2)—O(15)	122	121·8 (8)	C(8)—C(7)—C(18)	119	120·4 (3)†
N(3)—C(2)—O(15)	116	117·9 (5)	C(7)—C(8)—C(9)	120	121·7 (16)
C(2)—N(3)—C(4)	123	125·0 (9)	C(7)—C(8)—C(19)	121	120·8 (2)†
N(3)—C(4)—C(12)	115	115·0 (3)	C(9)—C(8)—C(19)	119	119·1 (2)†
N(3)—C(4)—O(16)	123	122·4 (2)	C(8)—C(9)—C(14)	120	119·6 (13)
C(12)–C(4)—O(16)	122	122·8 (3)	C(9)—C(14)–C(13)	122	119·8 (2)
C(4)—C(12)–C(11)	116	116·1 (10)	C(9)—C(14)–N(10)	122	122·2 (2)
C(11)–C(12)–N(5)	125	124·8 (5)	N(10)–C(14)–C(13)	117	117·8 (2)
C(12)–N(5)—C(13)	118	117·4 (2)	C(14)–N(10)–C(11)	122	121·4 (4)
N(5)—C(13)–C(14)	122	122·6 (2)	C(14)–N(10)–C(17)	118	119·7 (2)
C(14)–C(13)–C(6)	116	119·0 (4)	C(11)–N(10)–C(17)	120	118·8 (6)
N(5)—C(13)–C(6)	121	118·4 (3)	N(10)–C(11)–C(12)	116	115·9 (9)
C(13)–C(6)—C(7)	124	121·4 (5)	N(10)–C(11)–N(1)	118	118·9 (2)
C(6)—C(7)—C(8)	119	118·6 (3)	N(1)—C(11)–C(12)	126	125·2 (7)

*† Literature values are averages from 10-methylisoalloxazine (Wang & Fritchie, 1973) and 3-methyllumiflavin (Norrestam & Stensland, 1972) except for those marked †, which are from the latter. The uncertainties quoted in this column are the larger of (1) half the difference between the two literature values, or (2) the quoted standard deviation.

Table 5. *Least-squares planes*

m, *n*, and *p* are coordinates in Å relative to orthonormal vectors **m**‖**b** × **c***, **n**‖**b**, and **p**‖**c***. Atoms were weighted by their atomic numbers, except those in parentheses, which were given zero weight.

Plane I: $0{\cdot}7689m+0{\cdot}0425n+0{\cdot}6380p=5{\cdot}359$
Plane II: $0{\cdot}7557m+0{\cdot}0604n+0{\cdot}6521p=5{\cdot}424$
Plane III: $0{\cdot}7695m+0{\cdot}0342n+0{\cdot}6377p=5{\cdot}370$

Distances of atoms from planes (Å)

	Plane I	Plane II	Plane III		Plane I	Plane II	Plane III
N(1)	0·023	−0·013		C(11)	0·052	0·010	
C(2)	0·015	0·013		C(12)	0·013	−0·002	
N(3)	−0·031	−0·004		C(13)	0·022		0·009
C(4)	−0·023	0·000		C(14)	−0·006		−0·006
N(5)	0·032	(0·011)	0·014	O(15)	0·059	(0·064)	
C(6)	−0·008		−0·026	O(16)	−0·060	(−0·013)	
C(7)	0·014		0·001	C(17)	−0·115		
C(8)	0·014		0·013	C(18)	0·019		0·000
C(9)	0·002		0·008	C(19)	−0·007		−0·002
N(10)	−0·017	(−0·092)	−0·011				

Table 5 (*cont.*)

Plane IV: $0{\cdot}7269m+0{\cdot}0598n+0{\cdot}6841p=8{\cdot}651$

Distances (Å)

C(20)	−0·034	C(24)	0·000	C(28)	0·016
C(21)	0·013	C(25)	−0·025	C(29)	−0·006
C(22)	0·000	C(26)	−0·014	O(30)	(−0·096)
C(23)	0·021	C(27)	0·027	O(31)	(−0·018)

Plane V: $0{\cdot}2717m+0{\cdot}9160n+0{\cdot}2951p=1{\cdot}234$

Distances (Å)

C(32)	0·066	C(36)	0·004	C(40)	−0·050
C(33)	0·016	C(37)	0·043	C(41)	−0·013
C(34)	−0·024	C(38)	−0·032	O(42)	(0·161)
C(35)	−0·048	C(39)	−0·028	O(43)	(0·031)

O(43) involving internal hydrogen-bond acceptors to a mean value of 114° as previously observed (Wells *et al.*, 1974; Kuo, Dunn & Fritchie, 1974) is confirmed by this study, the observed values in this structure being 114·8 (8) and 115·4 (8)° respectively.

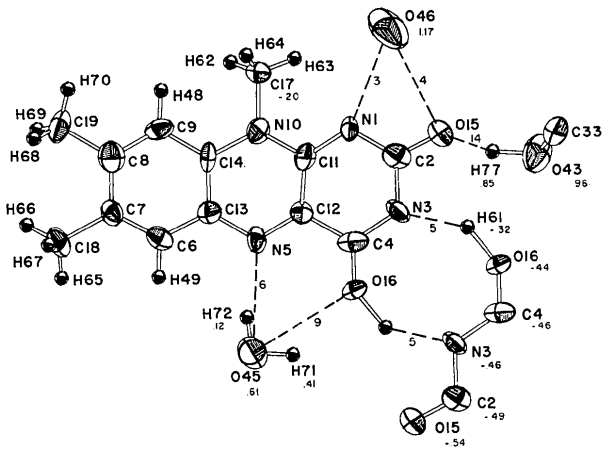
One highly unusual feature of the structure as tabulated, location of H(61) closer to O(16) of a neighboring molecule than to N(3), is not substantiated by the heavy-atom bond lengths. This atom forms part of a hydrogen-bonded, cyclic 'base pair'. It might in principle be possible for the flavins in a tightly hydrogen-bonded molecular pair to tautomerize from the usual 2,3 keto structure to an enol form, but both the C(2)–O(15) length of 1·238 (16) Å and the C(2)–N(3) length of 1·403 (16) Å indicate that this is not the case here. A final difference map, limited to data having $(\sin\theta/\lambda)\leq 0{\cdot}2$, and in which heavy atoms were removed, showed H(61) about 0·2 Å closer to N(3) than the position tabulated in Table 1, but still 1·6 Å from N(3). The peak was well defined and did not extend to a position 1·0 Å from N(3). The value of *R* for all data in this calculation was 0·110 rather than 0·081, indicating substantially correct positions for the hydrogen atoms located, but it must nevertheless be concluded from the anomaly concerning H(61), as well as the failure to locate three water protons, that the hydrogen positions are more uncertain than one would normally anticipate.

Deviations of the three aromatic molecules from planarity are reported in Table 5. The lumiflavin molecule as a whole, in common with all other oxidized flavins studied (Wells *et al.*, 1974; Kuo, Dunn & Fritchie, 1974; Wang & Fritchie, 1973, and references therein) is almost, but not quite planar. Unlike previously studied oxidized flavins, the lumiflavin in this structure shows neither a folding along N(5)–N(10), nor a longitudinal screwlike twist as the principal distortion. No systematic pattern is discernible in deviations from plane I, that through the whole molecule, although the large displacement of C(17) is not unexpected. Plane II shows that the entire dimethyldiaminobenzene group is essentially planar, and plane III, perhaps the most informative of several calculated through atoms of the diaminopyrimidinedione moiety, shows that the six atoms of the pyrimidine ring, as well as O(16) and N(5) are essentially coplanar, but O(15) and N(10) deviate significantly from this plane. The angle between planes

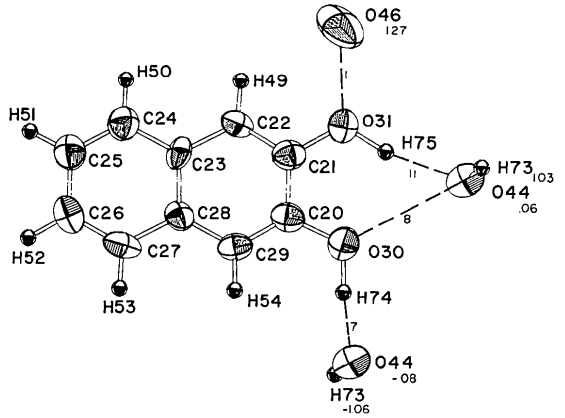
Table 6. *Probable intermolecular hydrogen bonds*

The numbers refer to Figs. 1 and 3.

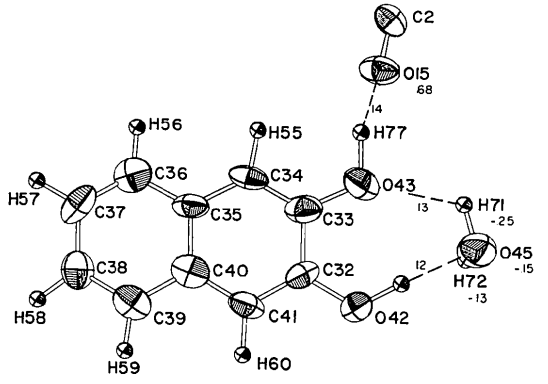
Bond	Donor	Acceptor	Length	Bond	Donor	Acceptor	Length
1	O(46)	O(31)	2·60 Å	8	O(44)	O(30)	3·05 Å
2	O(46)	O(15)	2·95	9	O(45)	O(16)	3·08
3	O(46)	N(1)	2·96	10	O(44)	O(45)	2·84
4	O(46)	O(15)	3·16	11	O(31)	O(44)	2·97
5	N(3)	O(16)	2·89	12	O(42)	O(45)	2·85
6	O(45)	N(5)	2·94	13	O(45)	O(43)	2·87
7	O(30)	O(44)	2·72	14	O(43)	O(15)	2·62



(*a*)



(*b*)



(*c*)

Fig. 1. Atomic numbering scheme and hydrogen bonds. (*a*) Lumiflavin. (*b*) π-Complexed naphthalene-2,3-diol, molecule *A*. (*c*) Naphthalene-2,3-diol, molecule *B*. The atoms are represented by 50% thermal ellipsoids. Dashed lines indicate probable hydrogen bonds, which are keyed by number to Table 6. Numbers by atom labels are heights in Å above or below planes parallel to the following pairs of vectors: (*a*) N(3)–C(12) and N(1)–C(4); (*b*) C(25)–C(21) and C(22)–C(29); (*c*) C(37)–C(33) and C(34)–C(41).

II and III is 1·9°, one of the smallest systematic distortions yet observed.

Plane IV shows that the π-complexed naphthalenediol molecule is planar, except for O(30), whereas plane V shows a bowing of naphthalenediol *B* with respect to its long axis, as well as a further significant departure of O(42) from this pattern. No reason can be seen in the molecular packing for this distortion. As Fig. 2 shows, this molecule occupies a principally hydrophobic pocket formed by the surrounding stacks of π-complexed molecules. Closest contacts are H(53)···C(32) = 3·0, H(67)···C(40) = 3·1, and H(65)···C(37) = 3·2 Å from below plane V; and H(70)···C(38) = 2·9 and H(50)···C(34) = 3·1 Å from above.

Hydrogen bonding

Figs. 1–3 show short contacts which can reasonably be considered hydrogen bonds and which are described in greater detail in Table 6. Langhoff & Fritchie (1970) observed several years ago that the area defined by N(5) and O(16) (or O[4] in standard flavin terminology), predicted by Bamberg & Hemmerich (1961) as the site of metal chelation in flavin–metal complexes, and since termed the primary chelate site (Fritchie, 1972*a*) in order to distinguish it from a secondary site of metal chelation at N(1)–O(15), is almost invariably occupied in the absence of a metal by a suitable hydrogen-bond donor. Water(45), the best-defined water molecule, occupies this site. It forms an ordinary donor bond to N(5), a bifurcated donor bond to O(16) and O(43), and acceptor bonds to H(73)–O(44) and H(76)–O(42).

Trus, Wells, Johnston, Fritchie & Marsh (1971), in reporting the first study of a flavin which was not protonated at N(1), recognized the probable strong affinity of the secondary chelate site for hydrogen-bond donors. This site is occupied by either a metal ion or a hydrogen bond in every structure reported since then (Fritchie, 1972*a*,*b*; Wade & Fritchie, 1973; Garland & Fritchie, 1974; Kuo, Dunn & Fritchie, 1974). In the present structure, the rather ill-defined water(46) occupies this site, lying nearly 1·2 Å from the flavin plane, and in position to form four potential hydrogen bonds (1–4 in Fig. 3). Since the water, holding only two protons, must serve as donor in all these bonds, the protons and, to some extent the oxygen, may be disordered in the crystal. This possible disorder may be responsible for the failure to locate these protons and in part for the large thermal parameters of O(46).

A further feature of flavin hydrogen bonding predicted by Langhoff & Fritchie to be nearly ubiquitous is an acceptor for N(3)H(61), and indeed the N(3)H(61)···O(16) base pairing found here serves to provide both this acceptor and a second donor for O(16). Wang & Fritchie (1973) have called attention to the frequent use of O(15) or (more commonly) O(16) and N(3)H(61) as a two-pronged donor–acceptor pair which can serve to bond with a suitable donor–acceptor pair in a second molecule. The extreme frequency with which

flavin–flavin base pairing is found suggests that pairing of flavins with the somewhat similar nucleic acid bases or barbiturates might be quite favorable.

The one prediction of Langhoff & Fritchie which has not been borne out by subsequent structures is a greater likelihood of hydrogen bonding at O(16) over O(15). O(16) in this structure forms two bonds, and O(15) perhaps three.

π-Complexing

As Fig. 2 reveals, this structure is broadly similar to the two previously studied complexes of neutral flavins with naphthalene-2,3-diol in that 1 : 1 stacks of alternating flavin and naphthalenediol molecules are arranged into lipid-like bilayers, with all hydrophilic groups pointing toward the centers of the bilayers (in this case, planes such as $x,y,\frac{1}{2}$). All the water molecules lie in this hydrophilic region and the primarily hydrophobic gaps between columns are filled by naphthalenediol molecules of type *B*.

The type *A* naphthalenediol molecules above and below a lumiflavin molecule are shown projected on the lumiflavin in Fig. 4. In comparing this π overlap with that found in the other two naphthalenediol structures, one is struck by the great variety present. The hydrophilic ends of the two molecules have the same polar sense in two structures but are opposed in the third. There is interaction on the part of upper and lower naphthalenediols with opposite ends of the flavin in two of the structures (those two which are orangish in color) but of both upper and lower donors with the phenylene-pyrazine region in the third (the yellow complex). Thus, unlike molecules or ions like tetracyanoquinodimethane which show great consistency in overlap from structure to structure (Fritchie & Arthur, 1966), flavins seem to belong to the larger category in which the strength of π interactions varies little with relative lateral movement of the donor and acceptor.

Some common features are observed in overlap patterns, however. First, overlap is rather extensive in all three complexes, and secondly, the hexagons of donor and acceptor remain in parallel orientation. This symmetry of donor–acceptor overlap is preserved in some of the complexes of protonated flavins as well (Bear, Waters & Waters, 1973; Karlsson, 1972) but partly destroyed in others (Langhoff & Fritchie, 1970; Tillberg & Norrestam, 1972), and is totally absent in the neutral riboflavin complex with the less symmetrical aromatic system of 5′-deoxy-5′-bromoadenosine (Voet & Rich, 1971). Finally, the general rule stated by Wang & Fritchie (1973) and by Wells *et al.* (1974), that shorter interactions involve the pyrimidine ring more often than the phenylene ring, is not followed in this structure, partly because of the non-parallelism of the donor and the acceptor, which are mutually inclined at an angle of 3·7°. Because of this non-parallelism, the closest approaches to the flavin plane of the upper molecule shown in Fig. 4 involve O(30) at 3·11, O(31) at 3·26, and C(20) at 3·28 Å, all overlapping the pyrazine or phenylene rings. The closest approaches of the lower molecule involve C(25) at 3·26, C(24) and C(26) at 3·29, and C(27) at 3·35 Å, again overlapping primarily the pyrazine ring.

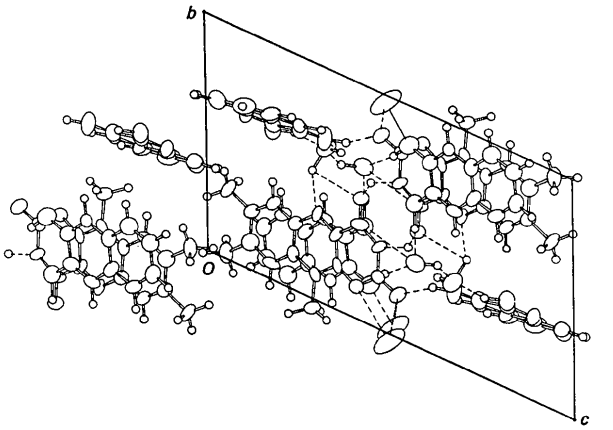


Fig. 2. [100] projection. Hydrogen bonds are dashed. Intramolecular hydrogen bonds are omitted.

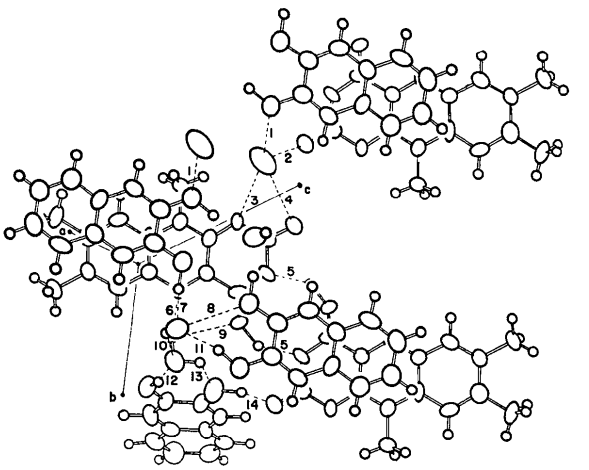


Fig. 3. A portion of the structure projected approximately onto the flavin plane, showing hydrogen bonds. Numbers refer to Table 6.

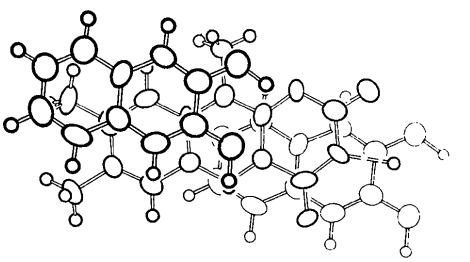


Fig. 4. Lumiflavin–naphthalenediol π overlap.

In summary, it seems that favorable apoprotein binding sites for flavins will provide maximal hydrogen-bonding opportunities for three principal regions of the flavin: acceptor sites at N[1]–O[2] and O[4]–N[5], and a donor site at N[3]. π-overlap with aromatic enzyme constituents or substrates does not seem at this point relatable to specific features or regions of the isoalloxazine nucleus, except that extensive overlap is favored, and point π-donors such as iodide seem to interact specifically with the C(11)–C(12) or C[10a]–C[4a] region (Kierkegaard *et al.*, 1971).

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