Crystal Structure of the Yellow Molecular Complex Lumiflavin–Bis(naphthalene-2,3-diol)*

By Joe L. Wells,† Benes L. Trus,‡§ Robert M. Johnston,† Richard E. Marsh‡ and Charles J. Fritchie Jr†

*Richardson Chemical Laboratories, Tulane University, New Orleans, Louisiana 70118, U.S.A. and
A. A. Noyes Laboratories, California Institute of Technology, Pasadena, California 91109, U.S.A.*

The only known molecular complex of a flavin which shows no apparent color change from that of the isolated flavin is nonetheless characterized by pillars of alternating flavin and naphthalenediol molecules running parallel to b, which involve extensive π overlap. In agreement with the lack of color change, intermolecular spacings are rather large, 3·41 Å and 3·48 Å. A second naphthalenediol molecule is highly tilted with respect to the stacked molecules but takes part in an extensive hydrogen-bonding system that binds the columns into sheets parallel to (100). The crystals are monoclinic, $P2_1/c$, with $a = 20·78$ (2), $b = 7·24$ (1), $c = 20·15$ (2) Å, $\beta = 116·19$ (5)°, $Z = 4$, $\varrho_{obs} = 1·40$ (1), $\varrho_{calc} = 1·402$ (3) g cm^{-3}. The final R index is 0·048, based on 1747 counter-measured reflections.

Introduction

The flavocoenzymes, which are the nonprotein portions of flavoenzymes, are widely involved in oxidation-reduction processes in the cell. The two forms most commonly found in biological systems are flavin mononucleotide (FMN or riboflavin 5'-phosphate) and flavin adenine dinucleotide (FAD), whose molecular structures are given in Fig. 1. The isoalloxazine or flavin nucleus, which is common to all forms, is the site of electron exchange and is shown in the air-stable, fully oxidized or quinoid form. It is reduced enzymatically by one or two electrons to either the semiquinoid or the hydroquinoid state. Since many of the substrates, including reduced pyridine nucleotide, DPNH, are aromatic or quasi-aromatic, it is possible that the electron interchange reaction occurs by formation of an intermediate, transient, charge-transfer complex (Szent-Gyorgyi, 1960; Cilento & Zinner, 1968; McCormick, 1968).

To study the charge-transfer complexing properties and also the hydrogen bonding properties of the flavin nucleus, we undertook this study. Naphthalene-2,3-diol was chosen as π-complex donor because of the large amount of information available on phenolic complexes (Tollin, 1968) and because of the possible *in vivo* interaction of flavins with phenols such as tyrosine and Coenzyme Q (ubiquinone). A preliminary description of this structure has appeared (Trus, Wells, Johnston, Fritchie & Marsh, 1971).

Experimental

Slow evaporation of a 1:1 acetone water solution of naphthalene-2,3-diol and lumiflavin (with the former

in ten to one hundred times greater molar concentration) generally yielded either a red oil or reddish-orange crystals of lumiflavin bis(naphthalene-2,3-diol) trihydrate (Fritchie & Johnston, 1972) but in one case also gave about a half dozen yellow crystals shown by this investigation to be anhydrous lumiflavin–bis-(naphthalene-2,3-diol), $C_{13}H_{12}N_4O_2 \cdot 2C_{10}H_8O_2$.

The crystals were nicely formed parallelepipeds ranging in size from 0·2 to 0·5 mm. One was mounted along the [010] direction, and served for the determination of unit-cell parameters and space-group symmetry. Equi-inclination Weissenberg photographs, $h0l$ through $h3l$, and an $hk0$ precession photograph revealed the crystal to be monoclinic with systematic extinctions ($h0l$ with l odd, $0k0$ with k odd) indicating space group $P2_1/c$. Initial unit-cell parameters were refined by a least-squares fitting to the $\sin^2\theta$ values for 15 reflections measured on a Picker diffractometer (Mo $K\alpha$ radiation; $\lambda = 0·7107$ Å). The refined lattice parameters with their estimated standard deviations are $a = 20·78$ (2), $b = 7·24$ (1), $c = 20·15$ (2) Å, and $\beta = 116·19$ (5)°. The density calculated for four lumiflavin and eight naphthalene-2,3-diol molecules per unit cell is 1·402 (3) g cm^{-3}, in excellent agreement with the experimental value of 1·40 (1) g cm^{-3}, measured by the flotation method.

A second parallelepipedal crystal, 0·07 mm by 0·16 mm in cross section and 0·26 mm in length, was mounted on its long axis, [010], for use in intensity measurement. A Picker four-circle card-controlled diffractometer operating in the θ–2θ scan mode collected the data, using Ni-filtered Cu $K\alpha$ radiation with a Tl-doped NaI scintillation counter serving as radiation detector. The pulse-height analyzer was set to accept about 90% of the Cu $K\alpha$ pulse distribution. All 2816 independent reflections having $2\theta \le 100°$ were scanned at a rate of 1° min^{-1}, with a scan range of 2° and background counts of 20 s at each end of the scan. Two periodically measured standard reflections showed less than ±1·5% variation. Those reflections for which the

---

peak scan rate exceeded 10000 c.p.s. were re-collected at a reduced tube current, and were scaled to the main set of data through measurements on a number of somewhat weaker reflections.

Of the reflections measured, 1753 were judged to be observed, using the criterion $I > 2\sigma_I$, where $\sigma_I = [C + (t_C/2t_B)^2(B_1 + B_2) + p^2 I^2]^{1/2}$. Here, C is the total scan count, $t_C = 120$ s, $t_B = 20$ s, B_1 and B_2 are the two background counts, $p = 0.02$, and I is the net intensity, $C - (t_C/2t_B)(B_1 + B_2)$. Lorentz (L) and polarization (P) corrections were used in deriving structure magnitudes, $|F_o|$, and corresponding standard deviations $\sigma_F = \sigma_I/(2.|F_o|.L.P)$. Because of the small crystal size and small absorption coefficient (8.20 cm^{-1}), no absorption corrections were applied to the data.

Structure solution and refinement

The iterative application of Sayre's equation (Sayre, 1952; equation 1·3) as applied to E's (Hauptman & Karle, 1953), using Long's (1965) program, led to a successful trial solution. Seven starting reflections – three whose signs were fixed to specify the origin and four whose signs were permuted through the 16 possible combinations – were selected according to a criterion proposed by Stanford (1973). Specifically, the rank of a reflection, $R(\mathbf{h})$, is expressed according to the relationship

$$R(\mathbf{h}) = |E_\mathbf{h}| N_\mathbf{h}^2 (1 - \sin^2 \theta_\mathbf{h}),$$

where $N_\mathbf{h}$ is the number of $\sum_2$ relationships involving reflection $\mathbf{h}$ with other reflections having large E values; reflections with high rank are good candidates for the initial phasing.

From the starting set of seven reflections, 485 additional reflections with E greater than 1·3 were assigned phases. Of the 16 starting sign combinations, one set converged quickly, resulted in an even distribution of signs, and had the highest consistency, 0·67. An E map based on this combination of signs clearly indicated positions for the three molecules – one lumiflavin and two naphthalenediols – in the asymmetric unit. Low-order structure factors derived from these positions

showed fair agreement with observed values; however, high-angle agreement was poor and the overall R index was a disappointing 0·70. After 20 structure-factor–difference-map cycles, when R had dropped to 0·50, it became apparent that a concerted shift of all three molecules, by an amount of about 0·5 Å and in a direction parallel to $\mathbf{a}$, had taken place. We applied a further shift of about 0·6 Å; the R index dropped immediately to 0·42, and least-squares refinement was initiated.

The least-squares refinement followed Hughes's (1941) procedure, with the quantity minimized being $\sum w(|F_o| - |F_c|)^2$, where $w = 1/\sigma_F^2$. All hydrogen atoms except those on C(17) were located in a series of difference maps; in the region expected for the latter there was a large poorly resolved area of positive density. These atoms were thus not included in any calculations. In the final stages of refinement, all atoms other than hydrogen were given anisotropic

Table 1. *Positional parameters*

Estimated standard deviations are given in parentheses.

	x	y	z
N(1)	0·0204 (2)	0·3294 (5)	−0·1754 (2)
C(2)	−0·0322 (2)	0·3381 (7)	−0·1528 (2)
N(3)	−0·0184 (2)	0·2917 (6)	−0·0798 (2)
C(4)	0·0463 (2)	0·2400 (7)	−0·0261 (2)
N(5)	0·1670 (2)	0·1839 (5)	−0·0001 (2)
C(6)	0·2895 (2)	0·1432 (7)	0·0320 (2)
C(7)	0·3476 (2)	0·1558 (7)	0·0168 (2)
C(8)	0·3359 (2)	0·2073 (7)	−0·0559 (2)
C(9)	0·2674 (2)	0·2463 (7)	−0·1086 (2)
N(10)	0·1409 (2)	0·2832 (7)	−0·1435 (2)
C(11)	0·0855 (2)	0·2836 (7)	−0·1246 (2)
C(12)	0·1030 (2)	0·2326 (7)	−0·0492 (2)
C(13)	0·2207 (2)	0·1865 (6)	−0·0212 (2)
C(14)	0·2094 (2)	0·2403 (6)	−0·0926 (2)
O(15)	0·0536 (1)	0·2033 (5)	0·0361 (2)
O(16)	−0·0938 (1)	0·3846 (5)	−0·1952 (1)
C(17)	0·3991 (2)	0·2169 (8)	−0·0748 (2)
C(18)	0·4214 (2)	0·1190 (8)	0·0774 (2)
C(19)	0·1258 (2)	0·3365 (9)	−0·2204 (2)
C(20)	0·7949 (2)	0·1638 (7)	0·4905 (2)
C(21)	0·8615 (2)	0·2096 (7)	0·5395 (2)
C(22)	0·8762 (2)	0·2733 (7)	0·6120 (2)
C(23)	0·8213 (2)	0·2886 (7)	0·6300 (2)
C(24)	0·7515 (2)	0·2417 (7)	0·5803 (2)
C(25)	0·6944 (3)	0·2566 (7)	0·6003 (2)
C(26)	0·6257 (2)	0·2091 (7)	0·5506 (3)
C(27)	0·6126 (2)	0·1477 (7)	0·4809 (3)
C(28)	0·6666 (2)	0·1322 (7)	0·4602 (2)
C(29)	0·7378 (2)	0·1792 (6)	0·5097 (2)
O(30)	0·9172 (2)	0·1882 (5)	0·5215 (2)
O(31)	0·9465 (2)	0·3199 (5)	0·6558 (2)
C(32)	0·3294 (2)	−0·1382 (7)	0·2429 (2)
C(33)	0·2572 (2)	−0·1536 (6)	0·2078 (2)
C(34)	0·2136 (2)	0·0031 (7)	0·1748 (2)
C(35)	0·2437 (2)	0·1742 (7)	0·1820 (2)
C(36)	0·3181 (2)	0·1958 (7)	0·2178 (2)
C(37)	0·3506 (2)	0·3669 (7)	0·2232 (2)
C(38)	0·4234 (2)	0·3844 (7)	0·2576 (2)
C(39)	0·4664 (2)	0·2313 (7)	0·2875 (2)
C(40)	0·4376 (2)	0·0620 (7)	0 2844 (2)
C(41)	0·3621 (2)	0·0394 (7)	0 2482 (2)
O(42)	0·2269 (1)	−0·3204 (4)	0·2041 (2)
O(43)	0·1414 (1)	−0·0312 (5)	0·1386 (1)
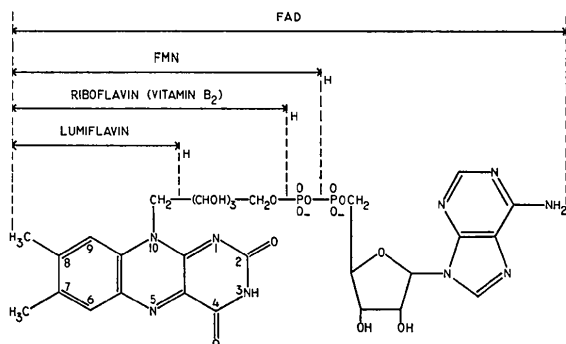
Fig. 1. The structures of several flavins.

Table 1 (cont.)

	x	y	z	B
H(44)	0·295 (2)	0·097 (6)	0·083 (2)	4·3 (1·0)
H(45)	0·261 (2)	0·281 (5)	−0·160 (2)	3·1 (1·0)
H(46)	−0·061 (2)	0·294 (6)	−0·072 (2)	6·1 (1·2)
H(47)	0·444 (2)	0·014 (6)	0·067 (2)	5·4 (1·1)
H(48)	0·449 (2)	0·229 (7)	0·089 (2)	7·7 (1·4)
H(49)	0·424 (2)	0·073 (7)	0·131 (2)	8·0 (1·4)
H(50)	0·162 (2)	0·395 (8)	−0·217 (3)	9·4 (1·6)
H(51)	0·134 (2)	0·237 (8)	−0·246 (3)	9·6 (1·6)
H(52)	0·078 (3)	0·369 (8)	−0·245 (2)	9·4 (1·5)
H(53)	0·788 (2)	0·110 (5)	0·438 (2)	3·9 (0·9)
H(54)	0·835 (2)	0·334 (5)	0·679 (2)	4·1 (1·0)
H(55)	0·703 (3)	0·317 (8)	0·659 (3)	11·2 (1·8)
H(56)	0·583 (3)	0·216 (7)	0·572 (3)	8·9 (1·5)
H(57)	0·561 (2)	0·111 (6)	0·439 (2)	4·9 (1·1)
H(58)	0·662 (2)	0·072 (6)	0·413 (2)	5·1 (1·0)
H(59)	0·951 (2)	0·245 (6)	0·548 (2)	6·7 (1·2)
H(60)	0·965 (2)	0·320 (7)	0·711 (3)	8·9 (1·5)
H(61)	0·357 (1)	−0·253 (5)	0·264 (2)	1·6 (0·8)
H(62)	0·214 (2)	0·281 (5)	0·157 (2)	2·8 (0·9)
H(63)	0·314 (2)	0·488 (6)	0·193 (2)	5·2 (1·0)
H(64)	0·444 (2)	0·509 (6)	0·259 (2)	5·6 (1·1)
H(65)	0·517 (2)	0·249 (5)	0·311 (2)	2·4 (0·8)
H(66)	0·469 (2)	−0·061 (6)	0·307 (2)	4·6 (1·0)
H(67)	0·179 (2)	−0·307 (6)	0·186 (2)	6·6 (1·2)
H(68)	0·121 (2)	0·084 (8)	0·099 (3)	10·0 (1·6)

thermal parameters; positional and isotropic thermal parameters for all hydrogen atoms except those on C(17) were also refined. The final R value ($R=$
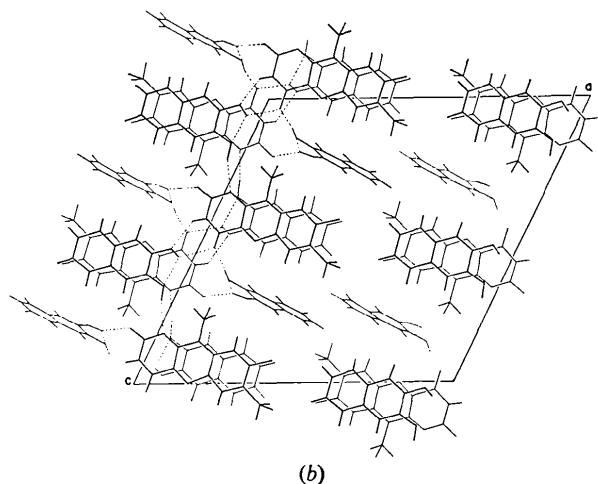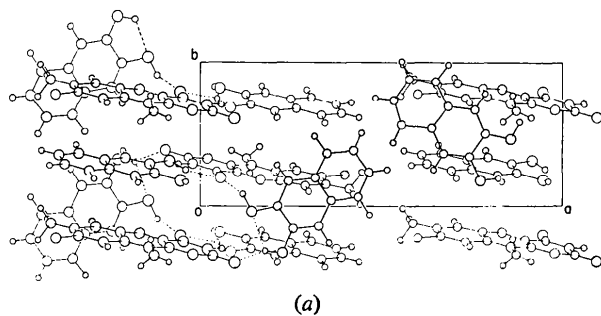

(a)

(b)

Fig. 2. (a) [001] projection. Hydrogen bonds are shown as dashed lines; the small circles are hydrogen atoms; (b) [010] projection.

Table 2. Anisotropic thermal parameters

Estimated standard deviations are given in parentheses.

All values are $\times 10^4$. The form of the temperature factor is $\exp\left[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl)\right]$.

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	21 (1)	227 (10)	20 (1)	−3 (6)	16 (1)	22 (6)
C(2)	25 (1)	216 (14)	26 (1)	−15 (7)	17 (2)	−1 (8)
N(3)	19 (1)	273 (11)	23 (1)	−4 (6)	19 (1)	8 (6)
C(4)	22 (1)	197 (13)	21 (1)	−2 (7)	16 (2)	10 (7)
N(5)	21 (1)	167 (10)	21 (1)	0 (5)	18 (1)	0 (6)
C(6)	24 (1)	188 (12)	22 (1)	−4 (7)	21 (2)	−11 (7)
C(7)	22 (1)	207 (13)	28 (1)	−5 (7)	22 (2)	−27 (8)
C(8)	27 (1)	195 (13)	27 (1)	−12 (7)	31 (2)	12 (8)
C(9)	30 (1)	211 (13)	21 (1)	9 (7)	22 (2)	13 (8)
N(10)	22 (1)	186 (10)	23 (1)	−8 (5)	25 (1)	6 (6)
C(11)	24 (1)	172 (12)	20 (1)	−16 (7)	19 (2)	−1 (7)
C(12)	23 (1)	163 (12)	20 (1)	−10 (7)	20 (2)	−5 (7)
C(13)	21 (1)	177 (12)	21 (1)	13 (7)	21 (2)	11 (7)
C(14)	17 (1)	189 (12)	20 (1)	1 (6)	17 (2)	3 (7)
O(15)	21 (1)	323 (10)	25 (1)	24 (5)	20 (1)	43 (5)
O(16)	23 (1)	298 (10)	25 (1)	2 (5)	11 (1)	53 (6)
C(17)	24 (1)	363 (17)	40 (1)	1 (8)	46 (2)	25 (9)
C(18)	23 (1)	292 (16)	33 (1)	18 (8)	19 (2)	−5 (9)
C(19)	29 (1)	408 (19)	22 (1)	25 (9)	26 (2)	48 (9)
C(20)	27 (1)	200 (13)	24 (1)	7 (7)	21 (2)	7 (8)
C(21)	26 (1)	212 (13)	29 (1)	22 (7)	30 (2)	20 (8)
C(22)	33 (1)	183 (13)	22 (1)	−5 (8)	21 (2)	25 (7)
C(23)	32 (1)	239 (14)	22 (1)	12 (8)	25 (2)	15 (8)
C(24)	27 (1)	200 (12)	24 (1)	23 (7)	27 (2)	24 (7)
C(25)	38 (1)	224 (14)	34 (2)	2 (8)	33 (2)	13 (8)
C(26)	37 (1)	267 (16)	41 (2)	6 (9)	40 (2)	12 (9)
C(27)	30 (1)	291 (16)	35 (2)	−22 (8)	25 (2)	4 (9)
C(28)	38 (2)	216 (14)	32 (2)	1 (8)	31 (2)	11 (8)
C(29)	22 (1)	171 (12)	22 (1)	−7 (7)	14 (2)	6 (7)
O(30)	32 (1)	323 (11)	34 (1)	−9 (5)	34 (1)	−16 (6)
O(31)	27 (1)	312 (10)	26 (1)	−29 (5)	12 (1)	−16 (6)
C(32)	20 (1)	176 (12)	30 (1)	16 (7)	27 (2)	6 (7)
C(33)	25 (1)	133 (11)	30 (1)	−4 (7)	29 (2)	−7 (7)
C(34)	18 (1)	250 (13)	19 (1)	−1 (7)	17 (2)	−1 (7)
C(35)	22 (1)	171 (12)	21 (1)	25 (7)	18 (2)	36 (7)
C(36)	21 (1)	158 (12)	21 (1)	12 (6)	20 (2)	−6 (7)
C(37)	25 (1)	190 (13)	29 (1)	−13 (7)	24 (2)	−12 (8)
C(38)	32 (1)	186 (13)	38 (2)	−18 (7)	36 (2)	−1 (8)
C(39)	20 (1)	238 (14)	32 (1)	−37 (7)	25 (2)	−34 (8)
C(40)	22 (1)	235 (14)	29 (1)	14 (7)	23 (2)	−21 (8)
C(41)	19 (1)	228 (13)	23 (1)	−4 (7)	22 (2)	−19 (8)
O(42)	21 (1)	174 (8)	45 (1)	4(5)	24 (1)	21 (5)
O(43)	19 (1)	234 (9)	29 (1)	−13 (5)	15 (1)	15 (5)

$\sum|F_o - F_c|/\sum|F_o|$) is 0·048, with six low-order reflections omitted. These reflections, which are suspected of extinction error, are (in each case we list hkl, $|F_o|$ and $|F_c|$): 200, 90·3, 107·4; 122, 156·2, 181·8; 121, 200·0, 230·3; 100, 104·8, 133·1; 102, 86·8, 128·4; 020, 315·8, 381·2.

The final atomic parameters are listed in Tables 1 and 2.* In the structure-factor and least-squares calculations the scattering factors used were those in *International Tables for X-ray Crystallography* (1962), except for hydrogen (Stewart, Davidson & Simpson,

* A table listing the final observed and calculated structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30353 (11pp.). Copies may be obtained through the Executive Secretary, International Union of Crystallography, 13 White Friars, Chester, CH1 1NZ, England.

1965). Calculations were performed either on the Tulane Computer Laboratory IBM 7044 computer or the California Institute of Technology IBM 360-55 computer. Major programs used were *GSET*4 by Prewitt (1964); *CRYM* by Duchamp, Westphal, Trus & Wang (1969); *BLSA*, *FOUR*, and *GENFOR* by Fritchie (1962); and *ORTEP* by Johnson (1965).

Discussion

Packing

Two views of the structure are shown in Fig. 2. It consists of stacks of alternating, approximately planar (see below) lumiflavin and naphthalenediol molecules running parallel to [010], with additional naphthalenediol molecules whose planes are highly inclined to those of the stacked molecules lying between the stacks and hydrogen-bonded to them, thus linking together adjacent stacks. There are also direct hydrogen-bond links between stacks, involving N(1), O(31), and H(60). The hydrogen bonded, π-complex network extends in two dimensions [parallel to the (100) plane] and creates a series of lipid-like bilayers with the hydrophilic ends of all three independent molecules pointed into the centers of the bilayers, and with only hydrophobic, van der Waals contacts between bilayers.

Details of the hydrogen-bonding system are shown in Fig. 3. As predicted from other studies (Langhoff & Fritchie, 1970; Wade & Fritchie, 1973), both the primary chelate site, N(5)-O(15), and the secondary site, N(1)-O(16), are occupied by -OH dipoles. Naphthalenediol A, that in the π-complex, forms a hydrogen bond with N(1), and naphthalenediol B serves as one of two donors to O(15). Interestingly, the hydrogen atoms of both internally hydrogen-bonded -OH groups appear to form bifurcated hydrogen bonds, one to O(16) and the other to O(15). The oxygen of the latter -OH group is also the receptor in the N(3)H(46)···O hydrogen bond, generating a cyclical two-molecule mutual donor–acceptor pair. All the oxygen and nitrogen atoms of the flavin molecule, excepting of course N(10), are thus quite generally active in hydrogen or ion binding and are likely to be used maximally for these purposes in flavoproteins. N(5) appears to be the weakest hydrogen-bond acceptor, but is the strongest site of binding for soft metals (Garland & Fritchie, 1974; Fritchie, 1973).

The lumiflavin molecule is not quite planar, as shown by data in Table 3. The major distortion, as in many other flavins (Wang & Fritchie, 1973, and references therein), is a folding along the N(5)-N(10) axis but, again in common with many other structures, such a description is much too simple. There is also a

Table 3. *Least-squares planes*

Each atom was given a weight equal to its atomic number, except those indicated by an asterisk, which were given zero weight. The angle between lumiflavin plane I and that of the naphthalenediol A molecule which lies in the same stack is 5·1°.

Equations of the planes based on the orthonormal coordinate system:

$$\mathbf{M}\|\mathbf{a},\ \mathbf{N}\|\mathbf{b},\ \mathbf{P}\|\mathbf{a}\wedge\mathbf{b},\ \text{are:}$$

Lumiflavin	I	$0{\cdot}0660m + 0{\cdot}9580n + 0{\cdot}2789p =$ 1·568 Å
	II	$0{\cdot}1132m + 0{\cdot}9568n + 0{\cdot}2677p =$ 1·672
	III	$0{\cdot}0124m + 0{\cdot}9585n + 0{\cdot}2849p =$ 1·243
Naphthalenediol A:		$-0{\cdot}0101m + 0{\cdot}9384n - 0{\cdot}3454p = -2{\cdot}085$
Naphthalenediol B:		$-0{\cdot}4807m + 0{\cdot}1098n + 0{\cdot}8603p =$ 1·355 .

|	Lumiflavin Deviation				Naphthalenediol A Deviation		Naphthalenediol B Deviation
	(I)	(II)	(III)				
N(1)	−0·035 Å	−0·015 Å		C(20)	0·013 Å	C(32)	−0·001 Å
C(2)	0·053	0·009		C(21)	0·008	C(33)	0·009
N(3)	0·075	0·000		C(22)	−0·007	C(34)	−0·018
C(4)	0·045	−0·001		C(23)	−0·004	C(35)	0·036
N(5)	−0·064	−0·006*	0·076 Å*	C(24)	−0·001	C(36)	0·027
C(6)	−0·035		−0·013	C(25)	−0·011	C(37)	0·019
C(7)	0·064		0·013	C(26)	−0·013	C(38)	−0·001
C(8)	0·081		0·000	C(27)	0·002	C(39)	−0·030
C(9)	0·024		−0·012	C(28)	0·013	C(40)	−0·013
N(10)	−0·049	0·071*	0·035*	C(29)	0·012	C(41)	0·004
C(11)	−0·037	0·016		O(30)	−0·038	O(42)	0·032
C(12)	−0·032	−0·007		O(31)	0·026	O(43)	−0·056
C(13)	−0·066		0·001				
C(14)	−0·025		0·012				
O(15)	0·078*	0·001*					
O(16)	0·104*	0·024*					
C(17)	0·152*		−0·012*				
C(18)	0·181*		0·082*				
C(19)	−0·044*						
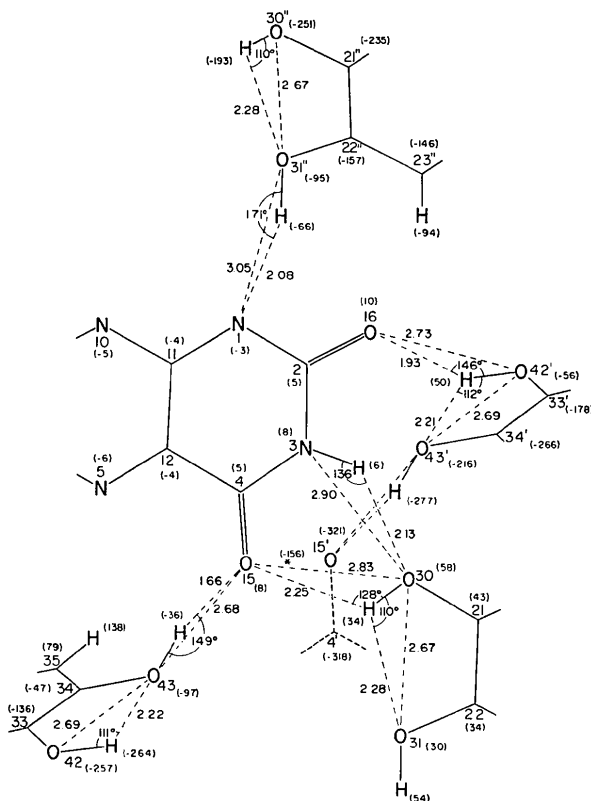
Fig. 3. Details of the hydrogen-bonding system. Figures in parentheses are distances from the mean flavin plane in Å × 10².

screwlike twist along the longitudinal axis of the molecule and other less systematic distortions.

Despite the lack of exact planarity of the flavin or naphthalenediol molecules, one can calculate average intermolecular separations in the rather extensive regions of intermolecular overlap shown in Fig. 4. These values are 3·48 Å for the upper naphthalenediol molecule shown and 3·46 Å for the lower. These rather large spacings are in agreement with the yellow color of the complex, which suggests little charge-transfer interaction.

It may also be noted that greater intermolecular separation is more often associated with overlap in the phenylene region of isoalloxazine than in the pyrimidine region. In the orange complex lumiflavin–bis-(naphthalene-2,3-diol) trihydrate (Fritchie & Johnston, 1972), average intermolecular spacings of 3·25 and 3·30 Å are found, with naphthalenediol overlapping the pyrazine and pyrimidine rings in the former case and the pyrazine and phenylene rings in the latter. Rather large spacings of 3·54 and 3·45 Å are seen in the complex riboflavin–5′-bromo-5′-deoxyadenosine trihydrate (Voet & Rich, 1971), the larger in this case involving overlap primarily with the pyrazine ring and the smaller with the phenylene. That factors other than distance and the gross pattern of overlap influence color is illustrated by the orange-brown color reported for this adenosine complex.

π-Complexes of protonated flavins are usually black or very deeply colored (Tollin, 1968), illustrating generally greater interaction, but the same trend

Table 4. *Comparison of distances*

	This structure $\sigma \simeq 0.012$ Å†	$(a)^*$ $\sigma \simeq 0.003$ Å	(b) $\sigma \simeq 0.012$ Å	(c) $\sigma \simeq 0.009$ Å	(d) $\sigma \simeq 0.008$ Å	(e)
N(1)—C(2)	1·356 Å	1·368 Å	1·387 Å	1·380 Å	1·408 Å	1·379 Å
C(2)—N(3)	1·413	1·410	1·389	1·362	1·379	1·379
N(3)—C(4)	1·349	1·362	1·375	1·359	1·385	1·366
C(4)—C(12)	1·447	1·483	1·485	1·466	1·489	1·466
C(12)—N(5)	1·309	1·300	1·297	1·305	1·299	1·308
N(5)—C(13)	1·358	1·372	1·378	1·355	1·376	1·368
C(13)—C(6)	1·395	1·406	1·398	1·411	1·411	1·415
C(6)—C(7)	1·371	1·371	1·372	1·362	1·348	1·387
C(7)—C(8)	1·422	1·421	1·416	1·409	1·415	1·407
C(8)—C(9)	1·380	1·376	1·414	1·375	1·362	1·389
C(9)—C(14)	1·379	1·397	1·422	1·399	1·419	1·411
C(14)—N(10)	1·374	1·380	1·415	1·390	1·386	1·379
N(10)—C(11)	1·365	1·360	1·356	1·329	1·338	1·363
C(11)—N(1)	1·327	1·303	1·324	1·362	1·364	1·323
C(11)—C(12)	1·446	1·446	1·445	1·440	1·422	1·450
C(13)—C(14)	1·405	1·405	1·420	1·424	1·407	1·414
C(8)—C(17)	1·525	1·502	1·509			
C(7)—C(18)	1·500	1·499	1·508			
N(10)—C(19)	1·486	1·466	1·489	1·511	1·491	
C(2)—O(16)	1·232	1·207	1·221	1·212	1·209	1·271
C(4)—O(15)	1·226	1·219	1·212	1·229	1·211	1·268

* (a) 3-Methyllumiflavin: Norrestam & Stensland (1972); (b) 9-bromo-3,7,8,10-tetramethylisoalloxazine: von Glehn, Kierkegaard & Norrestam (1970); (c) 10-methylisoalloxazine hydrobromide sesqui(naphthalene-2,7-diol)monohydrate: Langhoff (1968); (d) 10-methylisoalloxazine hydrobromide dihydrate: Trus & Fritchie (1969); (e) calculated for isoalloxazine: Fox, Nishimoto & Forster (1965).

† Standard deviations calculated from the uncertainties in Table 1 are about 0·005–0·007 Å, but these should probably be approximately doubled; see the text.
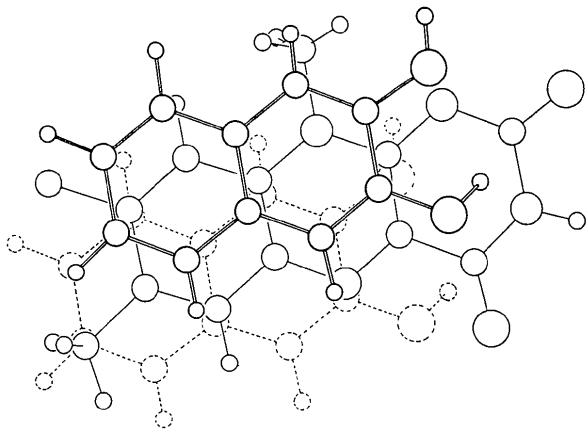
Fig. 4. Molecular overlap within a stack, viewed in a direction normal to the lumiflavin plane. The lower naphthalenediol molecule is indirectly hydrogen-bonded to the flavin, *via* a bridging naphthalenediol molecule.

regarding smaller spacing in the vicinity of the pyrimidine ring is seen. The black complex riboflavin dihydrobromide hydroquinone (Bear, Waters & Waters, 1970) contains a hydroquinone–phenylene overlap region having average spacing 3·35 Å and a hydroquinone–pyrimidine overlap region with spacing 3·28 Å. In the two protonated complexes lumiflavin hydrochloride hydroquinone and lumiflavin hydrobromide sesqui(hydroquinone) (Kierkegaard *et al.*, 1971), overlap of hydroquinone with the phenylene ring and with the pyrimidine ring is observed respectively, with average spacings of 3·51 and 3·27 Å. The colors of these two complexes are not reported.

Molecular geometry

The bond lengths and angles in the lumiflavin molecule are given in Figs. 5 and 6. A comparison of equivalent bond lengths in the two naphthalene-2,3-diol molecules in this structure, as well as comparison with other flavin molecules (Table 4), indicates that the positional standard deviations may be underestimated by a factor as large as 2. Such underestimation is common, and we assume in the following discussion that the actual uncertainties are double those reported in the tables. Only two compounds – 3-methyllumiflavin (Norrestam & Stensland, 1972), and 9-bromo-3,7,8,10-tetramethylisoalloxazine (von Glehn, Kierkegaard & Norrestam, 1970) – have been reported with which the lumiflavin molecule may be directly compared. Two additional accurately determined structures have been reported, however, which differ essentially only by protonation at N(1). Table 4 lists the bond lengths in these five structures and also those derived from the results of a molecular-orbital calculation by an empirical bond-order, bond-length correlation. Bond lengths in this compound and in 3-methyllumiflavin agree quite well. Agreement with the 9-brominated compound is likewise good and some of

the chief differences may be due to the bromination, as argued by Norrestam and Stensland. The bond lengths predicted by the molecular-orbital calculation also agree with the observed values, excepting the C=O distances, which consistently have high calculated values (Fox, Nishimoto & Forster, 1965).
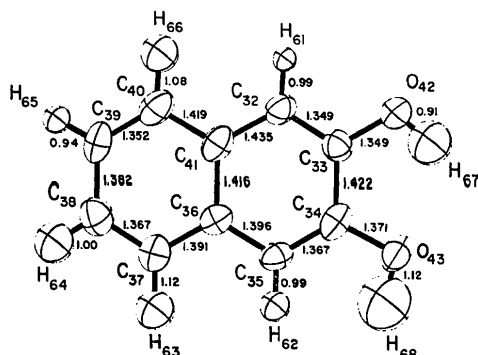
Where they are comparable, bond lengths in the un-
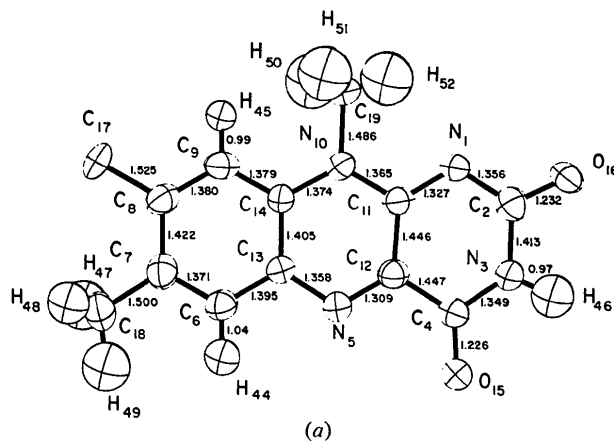
(a)
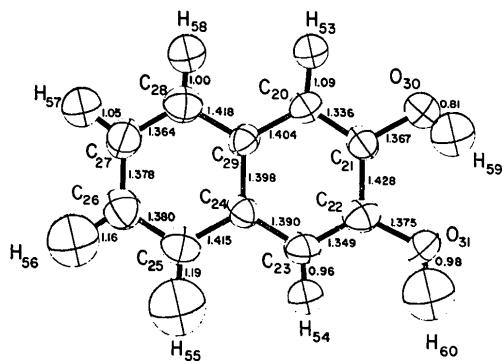
(b)

(c)

Fig. 5. Interatomic distances. (a) Lumiflavin. (b) Stacked naphthalenediol (molecule *A*). (c) Bridging naphthalenediol (molecule *B*). Calculated standard deviations average 0·006 Å for distances involving only heavy atoms and 0·04 Å for the remainder; however, see the text.

protonated and protonated compounds are also in agreement. The greatest differences, in the N(1)–C(11) and C(11)–N(10) bonds, are easily explained by the probable extra stability of the canonical structure having the charge at N(1).

Individual bond angles are not tabulated for all the structures, but the agreement is generally better than that for the distances. In particular, in comparison with 9-bromo-3,7,8,10-tetramethylisoalloxazine, the only significant differences occur at C(12)–C(4)–O(5) which has values of 124·7 (6) and 121·4 (7)°, and at N(3)–C(4)–O(15), which is 129·7 (6) and 124·4 (7)° in the two structures. These differences chiefly indicate movement of O(15) away from the region of the N(3)

methyl group in the tetramethyl compound. The one unusual but apparently consistent deviation in heavy-atom bond angles from 120° which occurs in the naphthalenediol molecules involves the oxygen which is an acceptor in the internal hydrogen bond. In each case, this atom bends about 5° toward the donor hydroxyl group.
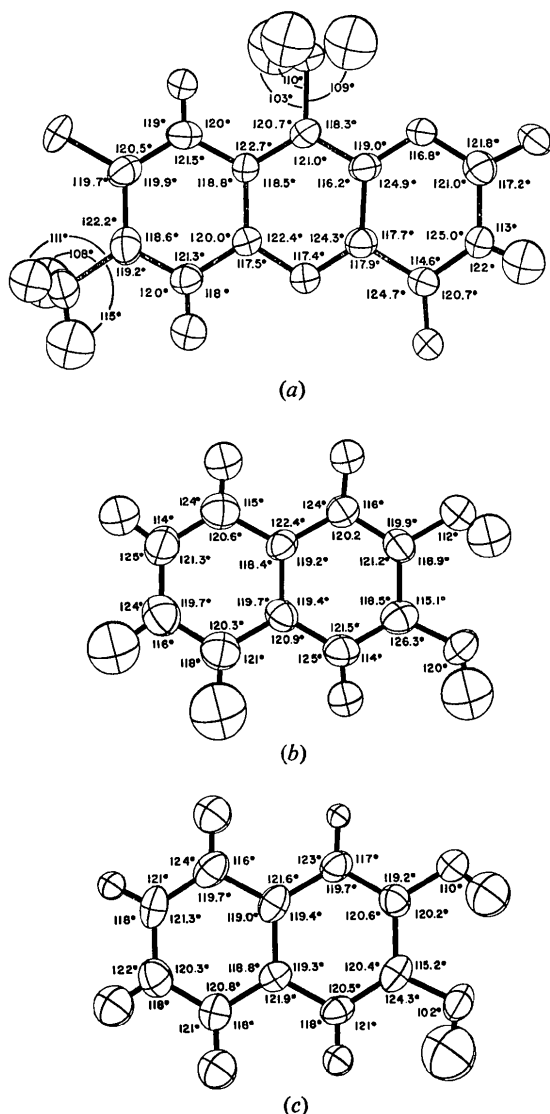
(a)

(b)

(c)

Fig. 6. Bond angles. (a) Lumiflavin. (b) Naphthalenediol A. (c) Naphthalenediol B. Calculated standard deviations are about 0·3° for angles involving only heavy atoms and 2·5° for others; however, these are probably underestimated. (See text.)

References

BEAR, C. A., WATERS, J. M. & WATERS, T. N. (1970). Chem. Commun. p. 702.

CILENTO, G. & ZINNER, K. (1968). Molecular Associations in Biology, edited by B. PULLMAN, p. 309. New York: Academic Press.

DUCHAMP, D. J., WESTPHAL, B. J., TRUS, B. L. & WANG, B.-C. (1969). Unpublished.

FOX, J. L., NISHIMOTO, K. & FORSTER, L. S. (1965). Biochim. Biophys. Acta, 109, 626–628.

FRITCHIE, C. J. JR (1962). Unpublished.

FRITCHIE, C. J. JR (1973). J. Biol. Chem. 248, 7516–7521.

FRITCHIE, C. J. JR & JOHNSTON, R. M. (1972). American Crystallographic Association Winter Meeting, Albuquerque. Abstract E11.

GARLAND, W. T. & FRITCHIE, C. J. JR (1974). J. Biol. Chem. 249. In the press.

GLEHN, M. VON, KIERKEGAARD, P. & NORRESTAM, R. (1970). Acta Chem. Scand. 24, 1490–1491.

HAUPTMAN, H. A. & KARLE, J. (1953). Solution of the Phase Problem. I. The Centrosymmetric Crystal. A.C.A. Monograph No. 3. Pittsburgh: Polycrystal Book Service.

HUGHES, E. W. (1941). J. Amer. Chem. Soc. 63, 1737–1752.

International Tables for X-ray Crystallography (1962). Vol. III, p. 202. Birmingham: Kynoch Press.

JOHNSON, C. R. (1965). ORTEP, ORNL-3794. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

KIERKEGAARD, P., NORRESTAM, R., WERNER, P. E., CSÖREGH, I., VON GLEHN, M., KARLSSON, K., LEIJONMARCK, M., RÖNNQUIST, O., STENSLAND, B., TILLBERG, O. & TORBJÖRNSSON, L. (1971). Flavins and Flavoproteins, Third International Symposium, edited by H. KAMIN, pp. 1–22. Baltimore: Univ. Park Press.

LANGHOFF, C. A. (1968). B.S. Hons. Thesis, Tulane Univ., New Orleans, La.

LANGHOFF, C. A. & FRITCHIE, C. J. JR (1970). Chem. Commun. pp. 20–21.

LONG, R. E. (1965). Ph.D. Dissertation, Univ. of California at Los Angeles, Los Angeles, California.

McCORMICK, D. B. (1968). Molecular Associations in Biology, edited by B. PULLMAN, p. 377. New York: Academic Press.

NORRESTAM, R. & STENSLAND, B. (1972). Acta Cryst. B28, 440–447.

PREWITT, C. T. (1964). GSET4, A Program for Computing Eulerian Orienter Angles, Central Research Laboratory, E.I. du Pont de Nemours and Co., Wilmington, Del. 19898.

SAYRE, D. (1952). Acta Cryst. 5, 60–65.

STANFORD, R. (1973). Private communication.

STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.

SZENT-GYORGYI, A. (1960). *Introduction to a Submolecular Biology*, p. 107. New York: Academic Press.

TOLLIN, G. (1968). *Molecular Associations in Biology*, edited by B. PULLMAN, p. 393. New York: Academic Press.

TRUS, B. L. & FRITCHIE, C. J. JR (1969). *Acta Cryst.* B**25**, 1911–1918.

TRUS, B. L., WELLS, J. L., JOHNSTON, R. M., FRITCHIE, C. J. JR & MARSH, R. E. (1971). *Chem. Commun.* pp. 751–752.

VOET, D. & RICH, A. (1971). *Proc. Natl. Acad. Sci. U.S.* **68**, 1151–1156.

WADE, T. D. & FRITCHIE, C. J. JR (1973). *J. Biol. Chem.* **248**, 2337–2343.

WANG, M. & FRITCHIE, C. J. JR (1973). *Acta Cryst.* B**29**, 2040–2045.

Structure Cristalline de La$_{32,66}$M$_{11}$S$_{60}$ (M=Mn, Fe)

PAR GASTON COLLIN* ET PIERRE LARUELLE†

U.E.R. des Sciences Pharmaceutiques et Biologiques de Paris V, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France

The structure of La$_{32·66}$M$_{11}$S$_{60}$ (M = Mn, Fe) has been studied by X-ray diffraction. Data have been collected on a G.E. diffractometer with balanced filters. Both crystals are monoclinic, space group *Bm*, with $a = 16·650$, $b = 10·906$, $c = 14·082$ Å, $\gamma = 102·58°$ for the manganese compound, and $a = 16·545$, $b = 10·845$, $c = 13·983$ Å, $\gamma = 102·91°$ for the iron compound. The crystal structure has been determined in two steps: first on the hexagonal quasi-substructure, space group $P6_3/mcm$, with $a' = 8·12$, $c' = 7·26$ Å, $V_{mono} = 6 \ V_{hex}$, which yielded a cell content of La$_6$Mn$_2$S$_{10}$; then on the monoclinic cells with refinement by full-matrix least-squares to a residual index of 0·072 (Mn) and 0·078 (Fe). The unit-cell contents are 32·80 La, 11 Mn, 60 S and 32·20 La, 11 Fe, 60 S. There are 12 non-equivalent lanthanum sites, four of which are not completely filled. The La coordination is 7–8. Four of the M sites are in octahedral coordination with S. The other two M sites have occupancies of 0·5 and give an M·M distance of approximately 2 Å.

L'existence d'un groupe de composés monocliniques de formule approximative L$_2$MS$_4$ avait été signalée par Patrie, Nguyen Huy-Dung & Flahaut (1968). Il s'agit d'un groupe restreint auquel appartiennent seulement cinq composés: ceux du lanthane avec le chrome, le manganèse et le fer, et ceux du cérium et du praséodyme exclusivement avec le fer.

Dans le cadre d'une étude générale des sulfures binaires de lanthanides et de métaux de transition, nous avions entrepris de déterminer la structure d'un représentant de cette famille en utilisant un cristal obtenu à partir d'une poudre de composition La$_2$FeS$_4$ (Collin, 1971). Mais un certain nombre de difficultés nous avaient à l'époque empêché de conclure et de proposer une formule et une structure tout à fait cohérentes pour ce composé.

Nous avions attribué ces difficultés au fait que le fer peut éventuellement prendre dans les sulfures deux états de valence différents (Fe^{II} et Fe^{III}) rendant impossibles des raisonnements fondés sur l'équilibre des charges.

Résultats expérimentaux

C'est pourquoi nous avons repris cette étude en préparant un cristal, à partir d'une poudre de composition La$_2$MnS$_4$, chauffée plusieurs heures à 1100°C dans un courant d'hydrogène sulfuré. En effet, dans ces conditions, le lanthane et le manganèse se trouvent toujours à l'état de La^{III} et Mn^{II} et les liaisons covalentes soufre–soufre sont instables, ce qui simplifie considérablement l'établissement des formules.

Le cristal obtenu était rouge-brun, transparent (caractéristiques de la présence exclusive des cations sous forme de La^{III} et Mn^{II}) et se présentait comme un parallélépipède tronqué de dimensions approximatives 50, 100 et 250 μm.

Nous avons mesuré pour les paramètres de la maille monoclinique:

$$a = 16,650 \ (6) \ \text{Å}$$
$$b = 10,906 \ (2)$$
$$c = 14,082 \ (2)$$
$$\gamma = 102,58° \ (3) \ .$$

La seule condition d'extinction systématique relevée: hkl, $h + l = 2n + 1$, correspond à trois groupes spatiaux *Bm*, *B2*, *B2/m*.

---

* Laboratoire de Chimie Minérale, Equipe de Recherche associée au CNRS n° 26.

† Laboratoire de Physique.