

## Studies on Flavin Derivatives. The Crystal and Molecular Structure of 9-Bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisalloxazine

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The crystal structure of 9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisalloxazine,  $C_{15}H_{17}BrN_4O_2$ , an example of a fully reduced flavin derivative, was deduced on the basis of three-dimensional X-ray diffractometer data. The final  $R$  value is 0.042 for the 1892 intensities used in the refinement. The atoms forming the alloxazine ring system of the molecule are close to two planes, one through the benzenoid ring and the other through the pyrimidinoid ring intersecting at an angle of about  $150^\circ$ . The molecular structure found confirms the hypothesis that the alloxazine ring system is non-planar in fully reduced 1,5-dihydroflavins.

### Introduction

Because flavins play an important role in biological processes, the physical and chemical properties of flavin derivatives have been intensively investigated in the last few years. For reviews on the chemistry and biological functions of flavins see *e.g.* Hemmerich, Veeger & Wood (1965), Penzer & Radda (1967) and Hemmerich, Nagelschneider & Veeger (1970).

The flavin redox system is usually described by three different redox states. The oxidized (quinone) state, the radical (semiquinone) state and the reduced (hydroquinone) state. In addition each of the three redox states is amphoteric. The redox and amphoteric activities of flavins are associated with changes of the alloxazine ring system of the flavins. 9-Bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisalloxazine (Fig. 1) is an example of a reduced neutral flavin derivative. A brief description of this structure investigation has been published earlier (Norrestam, Von Glehn, Hagman & Kierkegaard, 1969). This investigation is part of a research project concerning structural studies of flavin derivatives in different states of oxidation and protonation. A survey of the compounds investigated hitherto is given by Kierkegaard *et al.* (1970).

### Experimental

The yellow crystalline specimen used was prepared by L. Maron of this Institute. Details of the preparation are given by Maron (1969). A single crystal with dimensions  $0.2 \times 0.1 \times 0.1$  mm was selected and first examined by X-ray photographic methods, (oscillation and Weissenberg techniques). Weissenberg photographs showed that the crystal was monoclinic and that it was oriented along the  $b$  axis. The systematic extinctions indicated the possible space groups  $C2/c$  or  $Cc$ . More precise unit-cell parameters were obtained from manual measurements on a Siemens single-crystal X-ray diffractometer. A summary of the crystal data is given in Table

1. The X-ray intensities were collected on a Siemens AED (Automatischer Einkristall-Diffraktometer) equipped with a scintillation counter with pulse-height discrimination and with Ni-filtered Cu  $K$  radiation. The  $\theta$ - $2\theta$  scan technique was used and the background intensity was calculated as the average of the background intensities at each of the ends of the scan interval. The scan range used was  $1.5^\circ$ . Intensities were measured for all independent reflexions with  $\theta \leq 65^\circ$ . In order to keep the computer time used at a reasonable level, only the 1892 independent reflexions with  $\sigma(I_{net})/I_{net} \leq 0.10$  (based on counter statistics) were included in the structure refinement. Lorentz, polarization and absorption corrections ( $\mu = 45 \text{ cm}^{-1}$ ) were applied to the calculated net intensities.

Table 1. *Crystallographic data of 9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisalloxazine*

Lattice constants	$a = 15.680 (5) \text{ \AA}$ $b = 15.440 (5)$ $c = 12.845 (5)$ $\beta = 103.03 (5)^\circ$ $V = 3029.8 \text{ \AA}^3$
Cell volume	
Density (X-ray)	$d = 1.6 \text{ g.cm}^{-3}$
Molecules per unit cell	$Z = 8$
Absent reflexions	$hkl$ with $h+k = 2n+1$ $h0l$ with $l = 2n+1$ $0k0$ with $k = 2n+1$ $C2/c$ (or $Cc$ )
Space group	

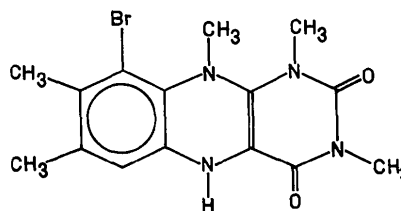


Fig. 1. Schematic drawing of 9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisalloxazine.

## Structure determination

To determine the most probable of the two possible space groups  $C2/c$  (centrosymmetric) and  $Cc$  (non-centrosymmetric) the normalized structure factors were calculated. The statistical averages and distributions of the  $|E|$ 's, given in Table 2, indicate that the electron-density distribution within the unit cell is largely centrosymmetric. For this reason, the space group  $C2/c$  was tentatively adopted for the structural study and fully supported by the outcome of the investigation.

Table 2. Statistical averages and distributions of normalized structure factors

	Experimental	Theoretical centrosymmetry	Theoretical non-centrosymmetry
$\langle  E  \rangle$	0.77	0.798	0.886
$\langle  E ^2 \rangle$	1.00	1.000	1.000
$\langle  E ^2 - 1 \rangle$	1.00	0.968	0.736
$ E  > 1$ (%)	31.4	32.00	37.00
$ E  > 2$ (%)	4.6	5.00	1.80
$ E  > 3$ (%)	0.3	0.30	0.01

The bromine atom position was determined from a three-dimensional Patterson synthesis, and refined by the method of least-squares. The  $R$  value ( $R = \sum |k|F_o| - |F_c| / \sum k|F_o|$ ) obtained at this stage was 0.42. Successive three-dimensional difference electron-density calculations enabled us to locate all the other 21 non-hydrogen atoms. Four cycles of full-matrix least-squares refinement of the atomic positions and temperature factors (anisotropic for bromine and isotropic for the other atoms) yielded an  $R$  value of 0.080. When anisotropic temperature factors for all 22 atoms were introduced into the refinement the  $R$  value was reduced to 0.055. From a subsequent difference electron-density map, reasonable positions for all 17 hydrogen atoms were obtained. A final full-matrix least-squares refinement (4 cycles), using anisotropic temperature factors for all the 22 non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms reduced the  $R$

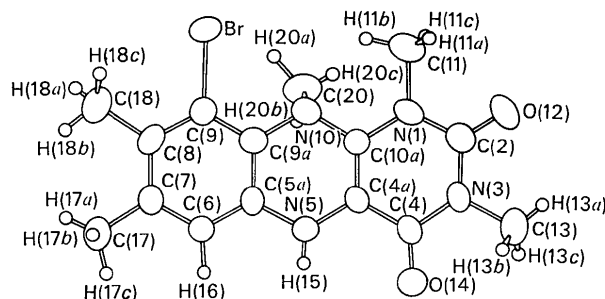


Fig. 2. Molecular conformation observed in the crystal structure of 9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisoalloxazine with atoms numbered for reference in the text, and the anisotropic thermal ellipsoids of the non-hydrogen atoms.

value to 0.042. The scattering factors corrected by the real part of the anomalous dispersion coefficient, used for bromine, carbon, nitrogen and oxygen were those given by Hanson, Herman, Lea & Skillman (1964), and for hydrogen that given by Stewart, Davidson & Simpson (1965). The weighting scheme recommended by Hughes (1941) was used in all the least-squares refinements. The positional and thermal parameters, with estimated standard deviations (e.s.d.'s) are given in Tables 3, 4 and 5. In Tables 6, 7 and 8 the interatomic distances and angles with e.s.d.'s are given. The observed and calculated structure factors are listed in

Table 3. Atomic coordinates ( $\times 10^4$ ) for the non-hydrogen atoms

	$x$	$y$	$z$
Br	405.6 (4)	3325.2 (5)	-160.8 (6)
N(1)	2113 (3)	3855 (3)	3543 (4)
C(2)	2678 (4)	3768 (4)	4517 (5)
N(3)	3558 (3)	3889 (3)	4520 (3)
C(4)	3887 (4)	4093 (4)	3641 (4)
C(4a)	3247 (4)	4182 (4)	2657 (4)
N(5)	3525 (3)	4366 (3)	1732 (3)
C(5a)	2994 (4)	4070 (4)	779 (4)
C(6)	3311 (3)	3903 (3)	-111 (4)
C(7)	2782 (4)	3568 (4)	-1036 (4)
C(8)	1902 (4)	3383 (4)	-1070 (4)
C(9)	1589 (4)	3569 (3)	-156 (4)
C(9a)	2107 (4)	3921 (3)	758 (4)
N(10)	1758 (3)	4134 (3)	1670 (4)
C(10a)	2387 (4)	4068 (3)	2618 (4)
C(11)	1178 (4)	3675 (6)	3493 (6)
O(12)	2435 (3)	3592 (3)	5307 (4)
O(13)	4192 (5)	3800 (5)	5554 (5)
O(14)	4672 (3)	4199 (3)	3721 (3)
C(17)	3180 (4)	3375 (5)	-1955 (4)
C(18)	1303 (4)	2985 (4)	-2040 (4)
C(20)	1282 (4)	4977 (4)	1546 (6)

Table 4. Anisotropic thermal parameters ( $\times 10^4$ ) for non-hydrogen atoms

$$T = \exp [ - (\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl) ]$$

	$\beta_{11}$	$\beta_{22}$	$\beta_{33}$	$\beta_{12}$	$\beta_{13}$	$\beta_{23}$
Br	31.2 (3)	58.5 (4)	76 (6)	-10.4 (5)	7.3 (5)	1.2 (9)
N(1)	35 (3)	53 (3)	62 (4)	-7 (4)	44 (5)	-10 (5)
O(2)	45 (3)	41 (3)	63 (5)	3 (5)	33 (6)	-9 (6)
N(3)	45 (2)	48 (2)	37 (3)	11 (4)	30 (4)	-3 (4)
C(4)	39 (3)	39 (3)	47 (4)	5 (4)	28 (5)	-14 (5)
C(4a)	38 (3)	38 (3)	44 (4)	-7 (4)	32 (5)	-6 (4)
N(5)	31 (2)	48 (2)	50 (3)	-13 (3)	18 (3)	-4 (3)
C(5a)	34 (2)	35 (2)	41 (3)	18 (4)	18 (4)	-11 (4)
C(6)	33 (2)	40 (2)	50 (3)	4 (3)	18 (4)	11 (4)
C(7)	40 (4)	37 (3)	44 (3)	10 (4)	15 (5)	11 (4)
C(8)	40 (3)	36 (2)	52 (4)	9 (4)	-4 (5)	4 (5)
C(9)	35 (2)	36 (2)	48 (4)	-1 (4)	9 (5)	13 (4)
C(9a)	32 (2)	33 (2)	57 (4)	6 (4)	18 (5)	12 (5)
N(10)	33 (2)	43 (2)	58 (3)	11 (3)	29 (4)	-5 (4)
C(10a)	37 (3)	38 (2)	53 (4)	-2 (4)	28 (5)	-9 (5)
C(11)	43 (3)	97 (4)	86 (5)	-17 (5)	54 (6)	-3 (7)
O(12)	66 (3)	80 (3)	61 (3)	-15 (5)	69 (5)	3 (5)
C(13)	63 (5)	58 (5)	47 (5)	23 (3)	20 (3)	-6 (4)
O(14)	35 (2)	74 (3)	59 (3)	-9 (3)	15 (4)	-5 (4)
C(17)	52 (3)	61 (3)	51 (3)	13 (5)	16 (5)	-4 (5)
C(18)	49 (3)	50 (3)	52 (3)	-2 (5)	-8 (5)	-4 (5)
C(20)	43 (3)	44 (3)	103 (5)	16 (4)	29 (6)	-19 (6)

Table 5. Atomic coordinates ( $\times 10^3$ ) for hydrogen atoms and isotropic thermal parameters ( $\text{\AA}^2$ )

	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i>
H(15)	404 (4)	428 (4)	173 (4)	4.84
H(16)	394 (4)	399 (4)	-4 (4)	4.60
H(11 <i>a</i> )	117 (5)	314 (5)	388 (6)	6.91
H(11 <i>b</i> )	95 (5)	351 (4)	272 (7)	7.09
H(11 <i>c</i> )	95 (5)	415 (6)	376 (6)	8.72
H(13 <i>a</i> )	393 (6)	382 (6)	604 (7)	8.25
H(13 <i>b</i> )	455 (4)	347 (5)	536 (6)	5.83
H(13 <i>c</i> )	454 (5)	423 (6)	562 (6)	7.30
H(17 <i>a</i> )	281 (6)	364 (5)	-254 (7)	8.51
H(17 <i>b</i> )	331 (6)	280 (6)	-200 (6)	8.91
H(17 <i>c</i> )	376 (6)	360 (6)	-178 (7)	9.42
H(18 <i>a</i> )	93 (6)	339 (5)	-244 (7)	7.96
H(18 <i>b</i> )	162 (6)	289 (5)	-258 (6)	6.80
H(18 <i>c</i> )	93 (6)	253 (6)	-198 (6)	9.41
H(20 <i>a</i> )	72 (4)	495 (4)	100 (5)	6.44
H(20 <i>b</i> )	164 (4)	549 (4)	144 (5)	6.08
H(20 <i>c</i> )	111 (4)	516 (5)	206 (5)	6.26

Table 6. Intramolecular bond distances

Br—C(9)	1.891 (6) Å	C(7)—C(17)	1.484 (8) Å
N(1)—C(2)	1.367 (8)	C(8)—C(18)	1.511 (8)
C(2)—N(3)	1.391 (8)	N(10)—C(20)	1.490 (8)
N(3)—C(4)	1.380 (7)	N(5)—H(15)	0.82 (6)
C(4)—C(4 <i>a</i> )	1.432 (8)	C(6)—H(16)	0.99 (6)
C(4 <i>a</i> )—N(5)	1.385 (6)	C(11)—H(11 <i>a</i> )	0.96 (7)
N(5)—C(5 <i>a</i> )	1.394 (6)	C(11)—H(11 <i>b</i> )	1.01 (8)
C(5 <i>a</i> )—C(6)	1.370 (7)	C(11)—H(11 <i>c</i> )	0.91 (9)
C(6)—C(7)	1.386 (7)	C(13)—H(13 <i>a</i> )	0.82 (9)
C(7)—C(8)	1.401 (8)	C(13)—H(13 <i>b</i> )	0.83 (7)
C(8)—C(9)	1.402 (8)	C(13)—H(13 <i>c</i> )	0.85 (8)
C(9)—C(9 <i>a</i> )	1.380 (8)	C(17)—H(17 <i>a</i> )	0.94 (9)
C(9 <i>a</i> )—N(10)	1.438 (7)	C(17)—H(17 <i>b</i> )	0.92 (10)
N(10)—C(10 <i>a</i> )	1.386 (7)	C(17)—H(17 <i>c</i> )	0.96 (10)
C(10 <i>a</i> )—N(1)	1.391 (7)	C(18)—H(18 <i>a</i> )	0.92 (8)
C(10 <i>a</i> )—C(4 <i>a</i> )	1.350 (8)	C(18)—H(18 <i>b</i> )	0.94 (7)
C(9 <i>a</i> )—C(5 <i>a</i> )	1.404 (8)	C(18)—H(18 <i>c</i> )	0.92 (9)
N(1)—C(11)	1.478 (8)	C(20)—H(20 <i>a</i> )	1.00 (7)
C(2)—O(12)	1.193 (8)	C(20)—H(20 <i>b</i> )	1.00 (7)
N(3)—C(13)	1.476 (8)	C(20)—H(20 <i>c</i> )	0.82 (7)
C(4)—O(14)	1.223 (7)		

Table 9. The atomic labels used are shown in Fig. 2. A list of the computer programs used throughout this investigation is given in Table 10. Most of the calculations were performed on an IBM 360/75 computer.

Br—C(9)—C(9 <i>a</i> )	118.1 (4)°	N(5)—C(5 <i>a</i> )—C(9 <i>a</i> )	117.6 (5)°	C(9 <i>a</i> )—N(10)—C(10 <i>a</i> )	111.9 (5)°
Br—C(9)—C(8)	119.0 (4)	N(5)—C(5 <i>a</i> )—C(6)	122.5 (5)	C(9 <i>a</i> )—C(5 <i>a</i> )—C(6)	119.9 (5)
N(1)—C(2)—N(3)	115.3 (5)	C(5 <i>a</i> )—C(9 <i>a</i> )—N(10)	120.3 (5)	C(10 <i>a</i> )—N(1)—C(11)	120.1 (5)
N(1)—C(2)—O(12)	122.4 (6)	C(5 <i>a</i> )—C(9 <i>a</i> )—C(9)	118.1 (5)	C(10 <i>a</i> )—N(1)—C(2)	122.9 (5)
C(2)—N(3)—C(13)	117.2 (5)	C(5 <i>a</i> )—C(6)—C(7)	121.7 (5)	C(10 <i>a</i> )—C(4 <i>a</i> )—N(5)	120.0 (5)
C(2)—N(3)—C(4)	125.6 (5)	C(6)—C(7)—C(8)	119.9 (5)	C(11)—N(1)—C(2)	116.9 (5)
N(3)—C(4)—O(14)	121.1 (5)	C(6)—C(7)—C(17)	118.6 (5)	O(12)—C(2)—N(3)	122.3 (6)
N(3)—C(4)—C(4 <i>a</i> )	115.3 (5)	C(7)—C(8)—C(9)	117.4 (5)	C(13)—N(3)—C(4)	117.2 (5)
C(4)—C(4 <i>a</i> )—C(10 <i>a</i> )	121.1 (5)	C(7)—C(8)—C(18)	122.2 (5)	O(14)—C(4)—C(4 <i>a</i> )	123.6 (5)
C(4)—C(4 <i>a</i> )—N(5)	118.9 (5)	C(8)—C(9)—C(9 <i>a</i> )	122.9 (5)	C(17)—C(7)—C(8)	121.5 (5)
C(4 <i>a</i> )—C(10 <i>a</i> )—N(1)	119.8 (5)	C(9)—C(9 <i>a</i> )—N(10)	121.6 (5)	C(18)—C(8)—C(9)	120.3 (5)
C(4 <i>a</i> )—C(10 <i>a</i> )—N(10)	122.0 (5)	C(9 <i>a</i> )—N(10)—C(20)	112.7 (5)	C(20)—N(10)—C(10 <i>a</i> )	113.4 (5)
C(4 <i>a</i> )—N(5)—C(5 <i>a</i> )	116.4 (5)	N(10)—C(10 <i>a</i> )—N(1)	118.1 (5)		

Table 7. Intramolecular bond angles

Table 8. Intermolecular distances less than 3.80 Å between non-hydrogen atoms

Code for symmetry-related atoms							
Super-script	Atom at			Super-script	Atom at		
none	<i>x</i>	<i>y</i>	<i>z</i>	v	- <i>x</i>	<i>y</i>	$\frac{1}{2}-z$
i	<i>x</i>	1- <i>y</i>	$\frac{1}{2}+z$	vi	<i>x</i>	<i>y</i>	1+ <i>z</i>
ii	$\frac{1}{2}-x$	$\frac{1}{2}-y$	1- <i>z</i>	vii	1- <i>x</i>	1- <i>y</i>	1- <i>z</i>
iii	$\frac{1}{2}-x$	$\frac{1}{2}-y$	- <i>z</i>	viii	<i>x</i>	1- <i>y</i>	$z-\frac{1}{2}$
iv	1- <i>x</i>	<i>y</i>	$\frac{1}{2}-z$				

Br—C(11 <sup>v</sup> )	3.668 (7) Å	C(5 <i>a</i> )—O(14 <sup>iv</sup> )	3.575 (7) Å
C(2)—C(5 <i>a</i> <sup>i</sup> )	3.696 (8)	C(5 <i>a</i> )—C(18 <sup>iii</sup> )	3.619 (8)
C(2)—C(6 <sup>i</sup> )	3.734 (8)	C(6)—O(14 <sup>iv</sup> )	3.294 (6)
C(2)—O(12 <sup>ii</sup> )	3.657 (8)	C(7)—C(9 <sup>iii</sup> )	3.677 (8)
N(3)—C(5 <i>a</i> <sup>i</sup> )	3.739 (7)	O(12)—C(20 <sup>i</sup> )	3.461 (8)
N(3)—C(6 <sup>i</sup> )	3.477 (7)	O(12)—O(12 <sup>ii</sup> )	3.480 (11)
C(4)—C(6 <sup>i</sup> )	3.690 (7)	O(12)—C(17 <sup>vi</sup> )	3.463 (7)
C(4)—C(18 <sup>iii</sup> )	3.786 (8)	O(14)—O(14 <sup>iv</sup> )	3.515 (8)
C(4 <i>a</i> )—C(18 <sup>iii</sup> )	3.546 (8)	O(14)—C(13 <sup>vii</sup> )	3.583 (9)
N(5)—O(14 <sup>iv</sup> )	3.022 (6)	C(18)—C(20 <sup>viii</sup> )	3.629 (9)
N(5)—C(13 <sup>i</sup> )	3.480 (9)		
N(5)—C(18 <sup>iii</sup> )	3.656 (8)		
C(5 <i>a</i> )—O(12 <sup>i</sup> )	3.732 (8)		

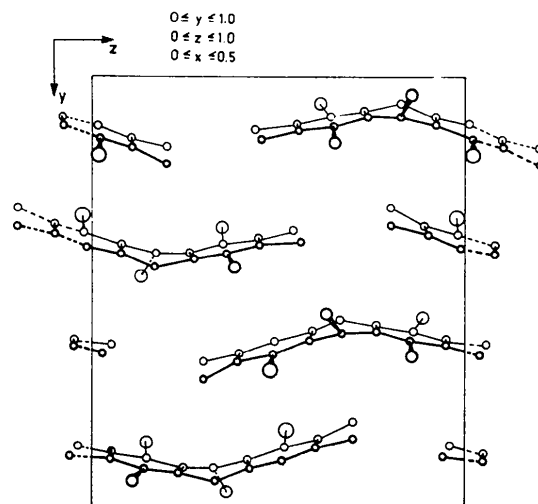
Fig. 3. A packing diagram, excluding hydrogen atoms, viewed along the *a* axis. Only half of the unit-cell content ( $0 \leq x \leq \frac{1}{2}$ ) is shown.

Table 9. Observed and calculated structure factors

Each group of three columns contains  $\kappa$ ,  $10|F_o|$  and  $10|F_c|$ , and is headed by the values of  $h$  and  $l$  common to the group.

$h$	$l$	$\kappa$	$10 F_o $	$10 F_c $
0	0	0	100	100
0	1	0	100	100
0	2	0	100	100
0	3	0	100	100
0	4	0	100	100
0	5	0	100	100
0	6	0	100	100
0	7	0	100	100
0	8	0	100	100
0	9	0	100	100
0	10	0	100	100
0	11	0	100	100
0	12	0	100	100
0	13	0	100	100
0	14	0	100	100
0	15	0	100	100
0	16	0	100	100
0	17	0	100	100
0	18	0	100	100
0	19	0	100	100
0	20	0	100	100
0	21	0	100	100
0	22	0	100	100
0	23	0	100	100
0	24	0	100	100
0	25	0	100	100
0	26	0	100	100
0	27	0	100	100
0	28	0	100	100
0	29	0	100	100
0	30	0	100	100
0	31	0	100	100
0	32	0	100	100
0	33	0	100	100
0	34	0	100	100
0	35	0	100	100
0	36	0	100	100
0	37	0	100	100
0	38	0	100	100
0	39	0	100	100
0	40	0	100	100
0	41	0	100	100
0	42	0	100	100
0	43	0	100	100
0	44	0	100	100
0	45	0	100	100
0	46	0	100	100
0	47	0	100	100
0	48	0	100	100
0	49	0	100	100
0	50	0	100	100
0	51	0	100	100
0	52	0	100	100
0	53	0	100	100
0	54	0	100	100
0	55	0	100	100
0	56	0	100	100
0	57	0	100	100
0	58	0	100	100
0	59	0	100	100
0	60	0	100	100
0	61	0	100	100
0	62	0	100	100
0	63	0	100	100
0	64	0	100	100
0	65	0	100	100
0	66	0	100	100
0	67	0	100	100
0	68	0	100	100
0	69	0	100	100
0	70	0	100	100
0	71	0	100	100
0	72	0	100	100
0	73	0	100	100
0	74	0	100	100
0	75	0	100	100
0	76	0	100	100
0	77	0	100	100
0	78	0	100	100
0	79	0	100	100
0	80	0	100	100
0	81	0	100	100
0	82	0	100	100
0	83	0	100	100
0	84	0	100	100
0	85	0	100	100
0	86	0	100	100
0	87	0	100	100
0	88	0	100	100
0	89	0	100	100
0	90	0	100	100
0	91	0	100	100
0	92	0	100	100
0	93	0	100	100
0	94	0	100	100
0	95	0	100	100
0	96	0	100	100
0	97	0	100	100
0	98	0	100	100
0	99	0	100	100
0	100	0	100	100

Table 9 (cont.)

$h$	$l$	$\kappa$	$10 F_o $	$10 F_c $
1	0	0	100	100
2	0	0	100	100
3	0	0	100	100
4	0	0	100	100
5	0	0	100	100
6	0	0	100	100
7	0	0	100	100
8	0	0	100	100
9	0	0	100	100
10	0	0	100	100
11	0	0	100	100
12	0	0	100	100
13	0	0	100	100
14	0	0	100	100
15	0	0	100	100
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18	0	0	100	100
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47	0	0	100	100
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92	0	0	100	100
93	0	0	100	100
94	0	0	100	100
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99	0	0	100	100
100	0	0	100	100

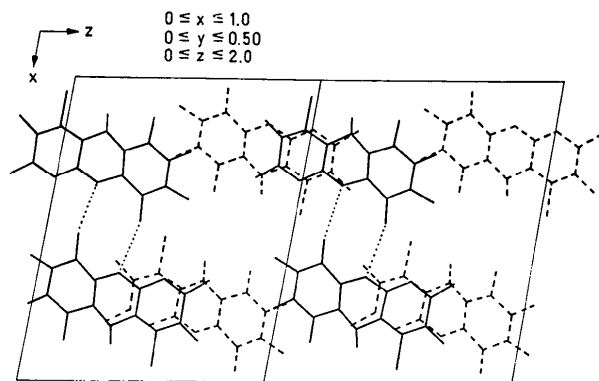


Fig. 4. A packing diagram, excluding hydrogen atoms, viewed along the  $b$  axis. Only half of the unit-cell content along  $b$  ( $0 \leq y \leq \frac{1}{2}$ ) is shown.

## Discussion

The crystal structure viewed along the  $a$  axis is shown in Fig. 3. The distances between adjacent molecules in the  $[010]$  and  $[001]$  directions are all larger than  $3.4 \text{ \AA}$  (*cf.* Table 8). Thus the molecules in the crystal structure are probably held together by conventional van der Waals forces in the  $[010]$  and  $[001]$  directions. In the  $[100]$  direction (Fig. 4) the molecules are connected by hydrogen bonds involving the hydrogen atom at the nitrogen atom N(5) in one molecule and the oxygen atom O(14) of another molecule, so as to form hydrogen-bonded dimers. This dimerization leads to a fairly short contact distance,  $3.294 \text{ \AA}$ , between the carbon atom C(6) in one molecule and the oxygen atom, O(14) of the other molecule in the dimers (Fig. 5).

The hydrogen-to-oxygen distance is  $2.23 \text{ \AA}$  and the N(5)–H...O(14) angle of  $165^\circ$ . The hydrogen-bonded dimers are separated from each other by about  $3.7 \text{ \AA}$ , *viz.* normal van der Waals contact distances. Since the

space group is centrosymmetric two conformations of the molecules (representing mirror images of each other) are present in the structure. The hydrogen-bonded dimers are made up of two molecules with the same conformation. Although the crystal structure contains both conformations of the molecule, it is likely (Hemmerich, Nagelschneider & Veeger, 1970) that reduced flavoenzymes especially when bound to a protein only appear in one of the conformations. Recent results from studies on flavoproteins (Rétey *et al.*, 1970; de Kok, Veeger & Hemmerich, 1970) indicate the existence of such so called 'flavinasymmetry' in flavoproteins.

In Fig. 6 the intramolecular distances are given together with approximate  $\pi$ -bond orders (within brackets). The bond orders were calculated from correlation functions between  $\pi$ -bond order and bond distance suggested by several authors (*e.g.* Roos & Skancke, 1967; Fischer-Hjalmars & Sundbom, 1968). Since the molecules are non-planar the bond orders should be treated only as approximate estimates. However, these bond orders agree fairly well with the commonly accepted  $\pi$ -bond scheme (*cf.* Fig. 1), for a reduced alloxazine system in the neutral state. Thus the highest  $\pi$ -bond order (0.9) within the ring system is that between C(4a) and C(10a).

The bond distances and angles in the benzenoid portion of the molecule, are within the range of values found in substituted benzene derivatives. The exocyclic C(17)<sub>sp3</sub>-C(7)<sub>sp2</sub> and C(18)<sub>sp3</sub>-C(8)<sub>sp2</sub> bond distances, 1.484 and 1.511 Å respectively, are somewhat shorter than the normal C<sub>sp3</sub>-C<sub>sp2</sub> bond distance which is about 1.52 Å (Sutton, 1958) found for example in toluene. This difference is probably not statistically significant within the experimental errors.

The endocyclic C-N bond distances within the molecules are all about 1.38 Å except for the C(9a)-N(10) bond distance 1.438 Å. As can be seen in Fig. 6, this bond will then have the lowest  $\pi$ -bond order within the ring system. This probably indicates a large degree of tetrahedral hybridization of the nitrogen atom N(10). The exocyclic C-N bond distances are all about 1.48 Å, which agrees well with the normal C-N single-bond distance 1.48 Å (Sutton, 1958).

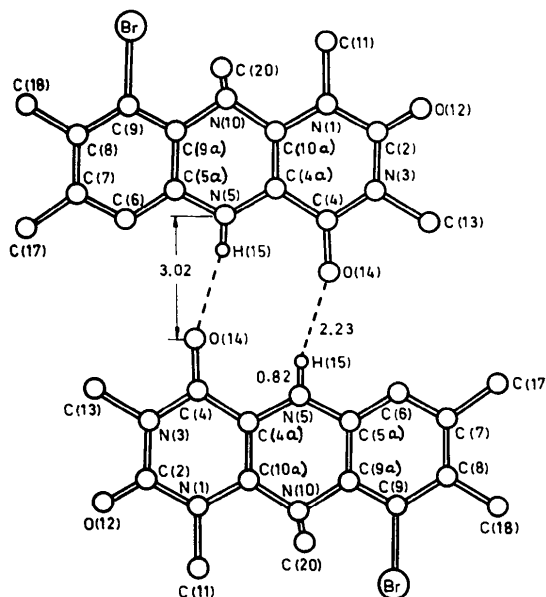


Fig. 5. The hydrogen-bonded dimers formed in the crystal structure. The hydrogen bonds involved in the dimerization are also indicated in Fig. 4.

Table 10. Computer programs used for the crystallographic calculations

Program name and function

*PIRUM*: Indexing of powder photographs and least-squares refinement of unit-cell parameters

*SIP*: Generation of steering paper tape for the Siemens AED

*SIMSA*: Interpretation of output on paper tape from the Siemens AED and evaluation of intensities

*DATAP 2*: Lp and absorption corrections. Preparative calculations for extinction correction

*DATA*: Reflection data handling

*DRF*: Fourier summations and structure-factor calculations

*FAME*: Wilson plot and calculation of *E* values

*LALS*: Full-matrix least-squares refinement of positional and thermal parameters and of scale factors

*DISTAN*: Calculation of interatomic distances and bond angles with estimated standard deviations

*INERT*: Calculation of least-squares plane and axes of inertia

*ORTEP*: Thermal-ellipsoid plot for crystal structure illustrations

*ACTA*: Editing of structure-factor tables

*SCF-OPSZDO*: Self-consistent field molecular orbital calculations

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The exocyclic C—O bond distances, 1.193 and 1.223 Å, confirm the suggestion by Dudley, Ehrenberg, Hemmerich & Müller (1964) based upon spectroscopic investigations, that reduced alloxazine species appear preferentially in the 'diketo' forms.

Thus, for a reduced flavin, the bonding situation in the pyrimidinoid part of the molecule should be rather similar to that of, for example, uracil derivatives. A comparison of the bond distances found in this study with those obtained by Stewart (1967) for uracil and by Reeke & March (1966) for 5-ethyl-6-methyl-uracil is given in Table 11. The agreement between the distances is satisfactory. The small differences are likely to be a result of substitution effects.

Table 11. Comparison of bond distances found in the pyrimidinoid part of 9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisalloxazine, with those obtained for uracil (Stewart, 1967) and for 5-ethyl-6-methyl-uracil (Reeke & March, 1966)

	This study	Uracil	5-Ethyl-6-methyluracil
N(1)—C(2)	1.367 (8) Å	1.371 (3) Å	1.360 (5) Å
C(2)—N(3)	1.391 (8)	1.376 (2)	1.363 (5)
N(3)—C(4)	1.380 (7)	1.371 (2)	1.381 (5)
C(4)—C(4a)	1.432 (8)	1.430 (3)	1.455 (5)
C(4a)—C(10a)	1.350 (8)	1.340 (2)	1.356 (5)
C(10a)—N(1)	1.391 (7)	1.358 (2)	1.377 (5)
C(2)—O(12)	1.193 (8)	1.215 (2)	1.229 (5)
C(4)—O(14)	1.223 (7)	1.245 (2)	1.230 (5)

The pyrazinoid ring of the molecule has the boat conformation, with the methyl group at N(10) and the hydrogen atom at N(5) both in axial boat positions. The entire ring system of the molecule can be described as consisting of two almost planar halves (*cf.* Table 12) folded about the N(5)—N(10) axis. The dihedral angle between the least-squares planes through the benzenoid ring and through the pyrimidinoid ring, including the N(5) and N(10) nitrogen atoms, is 149°. This angle is rather close to the dihedral angles found in other compounds with three-ring systems, where the atoms in the middle ring have a high degree of tetrahedral hybridization. Thus, the dihedral angles found in dihydroanthracene (Ferrier & Iball, 1954) and in phenothiazine (Bell, Blount, Briscoe & Freeman, 1968) are 145° and 153° respectively. Bending of the alloxazine ring system

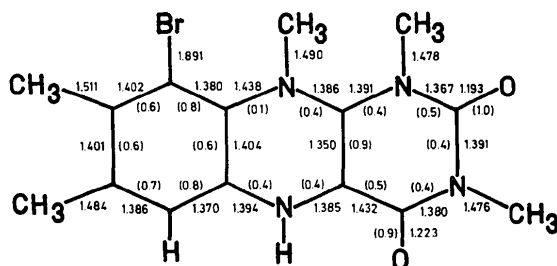


Fig. 6. Interatomic distances and estimated  $\pi$ -bond orders in 9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisalloxazine.

in a reduced flavin has been inferred by Dudley *et al.* (1964) on the basis of spectroscopic data. Semiempirical molecular orbital calculations of extended Hückel type (Hoffman, 1963) have also confirmed the bent molecular conformation for reduced flavins (Norrestam, Kierkegaard, Stensland & Torbjörnsson, 1969). In the present case the magnitude of the bending of the alloxazine ring is so great that it is unlikely that overcrowding alone could cause the effect. Furthermore, the molecular structures for oxidized flavin derivatives (Von Glehn, Kierkegaard & Norrestam, 1970), for which similar overcrowding effects would be expected, are almost coplanar.

Table 12. Deviations of the atoms from least-squares planes

The planes are of the form  $AX + BY + CZ = D$ , where  $X$ ,  $Y$  and  $Z$  are in Å units relative to the  $a^*$ ,  $b$  and  $c$  axes. The atoms indicated with asterisks were omitted from the calculations of the least-squares planes.

Plane I	Atom	Deviation
$A = -0.0912 \text{ Å}$	N(1)	-0.007 Å
$B = 0.9732$	C(2)	0.005
$C = 0.2113$	N(3)	0.000
$D = 6.3089$	C(4)	-0.002
	C(4a)	0.001
	C(10a)	0.004
	*N(5)	-0.032
	*N(10)	-0.021
	*O(12)	0.008
	*C(13)	0.011
	*O(14)	0.010

The e.s.d. of the first six atoms from this plane is 0.006 Å.

Plane II	Atom	Deviation
$A = -0.2408 \text{ Å}$	C(5a)	-0.014 Å
$B = 0.9250$	C(6)	0.000
$C = -0.2938$	C(7)	0.011
$D = 4.7418$	C(8)	-0.008
	C(9)	-0.005
	C(9a)	0.016
	*N(5)	-0.090
	*N(10)	0.068
	*C(17)	-0.023
	*C(18)	-0.052
	*Br	-0.039

The e.s.d. of the first six atoms from this plane is 0.015 Å.

The authors are indebted to Professor Peder Kierkegaard for his active and stimulating interest in this work, and for all facilities placed at their disposal. Thanks are also due to Lajos Maron for supplying the crystals used in the work and to Dr Don Koenig for his correction of the English of this paper. This investigation has been performed with financial support from the Tri-Centennial Fund of the Bank of Sweden and from the Swedish Natural Science Research Council. Permission for the use of the computer IBM 360/75 was granted by the Computer Division of the National Swedish Rationalization Agency. A research fellowship from the Stiftelsen Bengt Lundqvists Minne to one of us (R.N.) is gratefully acknowledged.

## References

- BELL, J. D., BLOUNT, J. F., BRISCOE, O. V. & FREEMAN, H. C. (1968). *Chem. Commun.* p. 1956.
- DE KOK, A., VEEGER, C. & HEMMERICH, P. (1970). In *Flavins and Flavoproteins*, Baltimore: Univ. Park Press.
- DUDLEY, K. H., EHRENBERG, A., HEMMERICH, P. & MÜLLER, F. (1964). *Helv. Chim. Acta*, **47**, 1354.
- FERRIER, W. G. & IBALL, J. (1954). *Chem. Ind.* p. 1296.
- FISCHER-HJALMARS, I. & SUNDBOM, M. (1968). *Acta Chem. Scand.* **22**, 607.
- HANSON, H. P., HERMAN, F., LEA, J. D. & SKILLMAN, S. (1964). *Acta Cryst.* **17**, 1040.
- HEMMERICH, P., VEEGER, C. & WOOD, H. C. S. (1965). *Angew. Chem.* **77**, 699.
- HEMMERICH, P., NAGELSCHNEIDER, G. & VEEGER, C. (1970). *FEBS-Letters*, **8**, 69.
- HOFFMAN, R. (1963). *J. Chem. Phys.* **39**, 1397.
- HUGHES, E. W. (1941). *J. Amer. Chem. Soc.* **63**, 1737.
- KIERKEGAARD, P., NORRESTAM, R., WERNER, P.-E., CSÖREGH, I., VON GLEHN, M., KARLSSON, R., LEIJONMARCK, M., RÖNNQUIST, O., STENSLAND, B., TILLBERG, O. & TORBJÖRNSSON, L. (1970). In *Flavins and Flavoproteins*, Baltimore: Univ. Park Press.
- MARON, L. (1969). In *Univ. Stockholm, Inorg. Chem.* DIS No. 32.
- NORRESTAM, R., VON GLEHN, M., HAGMAN, L. O. & KIERKEGAARD, P. (1969). *Acta Chem. Scand.* **23**, 2199.
- NORRESTAM, R., KIERKEGAARD, P., STENSLAND, B. & TORBJÖRNSSON, L. (1969). *Chem. Commun.* p. 1250.
- PENZER, G. R. & RADDA, G. K. (1967). *Quart. Rev.* **21**, 43.
- REEKE, G. N. & MARCH, R. E. (1966). *Acta Cryst.* **20**, 703.
- RÉTEY, J., SEIBL, J., ARIGONI, D., CORNFORTH, J. W., RYBACK, G., ZEJLEMAKER, W. P. & VEEGER, C. (1970). *Eur. J. Biochem.*
- ROOS, B. & SKANCKE, P. N. (1967). *Acta Chem. Scand.* **21**, 233.
- STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175.
- STEWART, R. F. (1967). *Acta Cryst.* **23**, 1102.
- SUTTON, L. E. (1958). *Tables of Interatomic Distances and Configuration of Molecules and Ions*. London: The Chemical Society.
- VON GLEHN, M., KIERKEGAARD, P. & NORRESTAM, R. (1970). *Acta Chem. Scand.* **24**, 1490.

*Acta Cryst.* (1972). B**28**, 440

## Studies on Flavin Derivatives The Crystal and Molecular Structure of 3-Methyl-lumiflavin

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The crystal structure of 3-methyl-lumiflavin (3,7,8,10-tetramethylisoalloxazine),  $C_{14}H_{14}N_4O_2$ , has been determined by direct methods from three-dimensional diffractometer data taken with Cu  $K\alpha$  radiation. The crystals are monoclinic, space group  $P2_1/c$ ,  $Z = 4$ ,  $a = 15.509$ ,  $b = 9.624$ ,  $c = 8.865$  Å and  $\beta = 107.88^\circ$ . The atomic parameters were refined by full-matrix least-squares to a final  $R$  of 0.047. The conformation of the molecules confirms that the isoalloxazine ring system in a neutral oxidized flavin derivative is almost planar, as has been suggested by spectroscopic data.

### Introduction

Flavin is the trivial name of the prosthetic group of a large class of respiratory enzymes, the flavoproteins, occurring in animals and plants. The simplest flavin compound, lumiflavin, is not known to occur in nature, but is formed from naturally occurring flavin species by photolysis under alkaline conditions. Furthermore, lumiflavin resembles the native coenzyme in its spectral, protolytic and redox properties. The chemistry and biological function of flavins have been reviewed by Penzer & Radda (1967) and by Hemmerich, Nagelschneider & Veeger (1970), *inter alios*.

The present determination of the structure of 3-methyl-lumiflavin,  $C_{14}H_{14}N_4O_2$  (Fig. 1), a neutral oxidized flavin derivative, is part of a research project concerning structural studies of flavin derivatives in different states of oxidation and protonation. A survey

of the flavin compounds previously investigated within this project is given by Kierkegaard *et al.* (1970).

### Experimental

The faintly yellow crystals used were supplied by L. Maron of this Institute. The selection of a suitable crystal and the determination of the possible space group were performed by X-ray photographic methods (oscillation and Weissenberg techniques). The systematic extinctions are characteristic of the space group  $P2_1/c$ . The crystal selected for collection of X-ray intensity data measured  $0.06 \times 0.10 \times 0.42$  mm, and was mounted along its long axis, parallel to the  $b$  axis.

More precise unit cell parameters,  $a = 15.509$  (5),  $b = 9.624$  (2),  $c = 8.865$  (2) Å and  $\beta = 107.88$  (3) $^\circ$ , were obtained from manual measurements on a Siemens single-crystal X-ray diffractometer. With four formula