# The Crystal Structure of 10-Methylisoalloxazine Hydrobromide Dihydrate\*

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The crystal structure of 10-methylisoalloxazine hydrobromide dihydrate,  $C_{10}H_9N_4O_2$ . HBr. 2H<sub>2</sub>O, has monoclinic symmetry  $P_{21}/c$ . Unit-cell constants are  $a=9.42\pm0.01$ ,  $b=11.81\pm0.01$ ,  $c=13.64\pm0.02$  Å,  $\beta=118.27\pm0.06^{\circ}$ . There are four molecules in the unit cell. All components of the structure lie in layers, with the layers grouped into pairs by hydrogen bonding. The nearly planar 10-methylisoalloxazine molecule is protonated at N(1) and bowed slightly on its long axis. N(1) and N(3) are hydrogen bond donors to water and bromide respectively; CO(4) is a hydrogen bond receptor.

## Introduction

Riboflavin (I, with R = D-ribityl and R' = methyl)



is coenzyme for a large family of flavoproteins, catalysts in biological redox reactions. The coenzyme, usually along with a metal atom, is present at the enzymic active site (Hemmerich, Müller & Ehrenberg, 1965), and binding to the metal through N[5] and CO[4]† is proposed (Hemmerich & Fallab, 1958; Hemmerich, 1964). It has been suggested that chargetransfer bonding between flavin and the substrate may be catalytically important (Szent-Gyorgyi, 1960). To clarify the mode of bonding of riboflavin to metal and to substrate, we have begun studying simple flavins in various molecular complexes. This paper, reporting the structure of 10-methylisoalloxazine hydrobromide dihydrate (I, R=methyl, R'=hydrogen), is the first of the series.

### **Experimental data**

Crystals were grown from hot concentrated HBr saturated with 10-methylisoalloxazine. Cooling produced transparent yellow needle-like gems with [010] as the needle axis.

The reciprocal-lattice symmetry and systematic absences, determined from the zero and first layer Weissenberg photographs of a crystal rotating about **b** and an 0kl precession picture, indicated that the space group is the monoclinic group  $P2_1/c$ . Preliminary lattice constants determined from the *b* axis rotation photograph and the *h0l* Weissenberg photograph were refined by the use of  $2\theta$  measurements made with a Picker diffractometer. The refined values are a= $9.42 \pm 0.01$ ,  $b=11.81 \pm 0.01$ ,  $c=13.64 \pm 0.02$  Å, and  $\beta=118.27 \pm 0.06^{\circ}$ . The density was determined to be 1.74 g.cm<sup>-3</sup> by flotation techniques, whereas it is calculated to be 1.72 g.cm<sup>-3</sup> with four formula units per cell.

Intensity data were measured on a Picker cardcontrolled four circle diffractometer with Zr-filtered Mo Ka radiation. Reflections with  $3^{\circ} \le 2\theta \le 40^{\circ}$  were scanned in the  $2\theta$  mode at a rate of 1° per minute, with a scan range of 2°. A twenty second background count was recorded on either side of the scan range. A pulse height analyzer adjusted to record about 90% of the Mo  $K\alpha$  pulse distribution was used in conjunction with a scintillation counter as a radiation detector. Two standards were determined approximately every four hours and served to measure any variation in the crystal or instrument. The standards decreased by an average of about 10% with time. There was no significant change in the appearance of reflections, although the crystal became slightly clouded. The crystal used had approximate dimensions  $0.54 \times 0.28 \times 0.42$  mm. Absorption and extinction corrections were not applied.

Altogether, 2354 reflections were scanned. Each reflection was assigned a standard deviation based on a combination of standard counting statistics in the integrated scan and in the background plus an uncertainty of 3% in the time-dependent scale factor. Squares of structure factor amplitudes,  $|F_o|^2$ , and their standard deviations were derived from the net intensities by application of the Lorentz and polarization (Lp) factors.

Any reflection for which the net value of  $|F_0|^2$  was less than twice the corresponding standard deviation was considered to be below an observational threshold.

<sup>\*</sup> Previously reported in part: Trus & Fritchie, Chem. Comm. (1968), p. 1486.

<sup>†</sup> Brackets are used to refer to the chemical numbering system, (I). Parentheses refer to the crystallographic system, Fig. 1.

The final data set contained 668 of these 'unobserved' reflections and 1686 observed reflections. For each of the observed reflections a standard deviation in  $|F_o|$  was derived by the standard propagation-of-error equation. Similar standard deviations were derived for the other reflections by use of the threshold value of  $|F_o|, 2\sigma_F$ . The unobserved reflections were omitted from all calculations, except for a comparison with their final calculated values.

#### Structure solution and refinement

The structure was solved by interpretation of the threedimensional Patterson function by the heavy atom method. A first Fourier synthesis, with  $R(=\Sigma | F_o F_c|\Sigma|F_o|=40\%$  based on signs determined from the location of the bromine atom alone and by use of all of the observed data, permitted location of a clear napthalene-like structure, addition of which lowered R to 36%. The two oxygen atoms and the methyl carbon atom were found in a second Fourier synthesis and lowered R to 31% after isotropic least-squares refinement (Hughes, 1941) of all the atoms. Addition of the four remaining ring carbon atoms produced an R of 25% after refinement. Location of the two oxygen atoms of the water molecules lowered R to 17%. After anisotropic refinement of bromine alone for three cycles, all atoms were refined anisotropically, lowering R to 7.3%. The aromatic hydrogen atoms and two water hydrogen atoms were found on a difference map calculated at this stage. With these added, the heavy atoms refined further to R = 6.2%. The methyl hydrogen atoms were then found on a difference map. Refinement of all parameters except the hydrogen temperature

#### Table 1. Positional and isotropic thermal parameters

Standard deviations are given in parentheses.

	<i>x</i> *	у*	<i>z</i> *	В
Br(1)	29169 (6)	12810 (4)	3061 (4)	
N(2)	1910 (4)	3261 (4)	2263 (3)	
C(3)	2863 (6)	4239 (4)	2686 (4)	_
N(4)	4459 (5)	4066 (3)	3449 (3)	—
C(5)	5179 (5)	3029 (4)	3882 (4)	_
C(6)	4115 (5)	2026 (4)	3398 (3)	
N(7)	4766 (4)	1036 (3)	3736 (3)	—
C(8)	3810 (6)	100 (4)	3276 (4)	
C(9)	4494 (6)	-987 (4)	3609 (4)	
C(10)	3592 (6)	-1915 (4)	3139 (4)	
C(11)	1966 (5)	-1806 (3)	2306 (3)	
C(12)	1248 (6)	-773 (4)	1994 (4)	
C(13)	2178 (5)	214 (4)	2478 (3)	
N(14)	1516 (4)	1286 (3)	2177 (3)	
C(15)	2473 (5)	2189 (4)	2597 (4)	_
C(16)	-213(6)	1420 (4)	1335 (4)	
O(17)	2282 (5)	5168 (3)	2377 (3)	_
O(18)	6580 (4)	2953 (3)	4578 (3)	
O(19)	-1136 (4)	3899 (3)	385 (3)	_
O(20)	1106 (5)	3758 (3)	- 586 (3)	
H(21)	98 (6)	331 (4)	182 (4)	5·3 (1·4) Å <sup>2</sup>
H(22)	530 (6)	467 (4)	393 (4)	4.6 (1.3)
H(23)	559 (5)	-103(4)	420 (3)	3.4 (1.1)
H(24)	394 (5)	-258 (3)	334 (3)	3.4 (1.1)
H(25)	104 (6)	- 259 (4)	181 (4)	5.4 (1.4)
H(26)	15 (5)	-65(3)	143 (3)	3.0 (1.1)
H(27)	-83(6)	95 (4)	152 (4)	5.6 (1.4)
H(28)	-35 (8)	134 (5)	72 (5)	8.0 (1.6)
H(29)	- 60 (6)	215 (4)	144 (4)	4.9 (1.3)
H(30)	-128(6)	456 (4)	45 (4)	4.1 (1.3)
H(31)	- 222 (7)	367 (5)	0 (5)	8.9 (1.6)
H(32)	34 (5)	377 (4)	- 39 (4)	3.8 (1.2)
H(33)	159 (7)	292 (6)	-30(5)	8.5 (1.6)

\* Positional parameters and standard deviations multiplied by  $10^5$  for bromine,  $10^3$  for hydrogen and  $10^4$  for remaining atoms.

#### Table 2. Anisotropic thermal parameters

Standard deviations are given in parentheses. Because no absorption correction was made, systematic errors may be present which exceed the usual statistical errors. The thermal expression has the form

$$-[B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{12}hk + B_{13}hl + B_{23}kl].$$

Parameters are multiplied by 10<sup>5</sup> for bromine and 10<sup>4</sup> for other atoms.

	$B_{11}$	B <sub>22</sub>	B <sub>33</sub>	<i>B</i> <sub>12</sub>	$B_{13}$	B <sub>23</sub>
Br(1)	1193 (6)	616 (4)	561 (3)	103 (11)	757 (6)	2 (8)
N(2)	95 (6)	51 (3)	48 (3)	21 (7)	36 (6)	7 (5)
C(3)	151 (7)	40 (3)	54 (3)	-2(9)	99 (7)	-6 (6)
N(4)	103 (6)	50 (3)	51 (3)	-24(7)	52 (6)	-15(5)
C(5)	84 (6)	59 (4)	41 (3)	-18 (9)	38 (6)	-3 (6)
C(6)	114 (6)	43 (4)	35 (3)	2 (8)	71 (6)	-4 (5)
N(7)	96 (5)	59 (3)	37 (2)	8 (7)	50 (5)	4 (5)
C(8)	120 (7)	46 (4)	43 (3)	6 (9)	75 (6)	4 (6)
C(9)	125 (7)	59 (4)	45 (3)	20 (9)	58 (7)	11 (6)
C(10)	164 (8)	50 (4)	62 (3)	30 (10)	104 (8)	20 (6)
C(11)	179 (8)	52 (4)	59 (3)	-9 (10)	106 (8)	-7 (7)
C(12)	108 (7)	63 (4)	52 (3)	-12 (10)	64 (7)	7 (6)
C(13)	114 (6)	36 (3)	36 (3)	-3 (8)	67 (6)	2 (5)
N(14)	89 (5)	51 (3)	39 (2)	-10 (7)	51 (5)	2 (5)
C(15)	108 (6)	57 (4)	37 (3)	-5 (9)	60 (6)	3 (6)
C(16)	87 (6)	64 (4)	71 (4)	6 (10)	40 (8)	9 (7)
O(17)	171 (6)	49 (3)	84 (3)	8 (7)	68 (7)	20 (5)
O(18)	109 (5)	61 (3)	71 (3)	-19 (7)	23 (6)	-1 (5)
O(19)	136 (6)	56 (3)	84 (3)	4 (7)	52 (6)	21 (5)
O(20)	185 (6)	79 (3)	107 (3)	24 (9)	139 (6)	9 (6)

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1 175 -181 2 517 512 1 2 60	0 894 -992 1 855 -693 2 870 -715	1 100 -72	6 246 250 9 262 -265 10 131 144	-1 121 -135 -2 197 301 -2 197 200	1 310 347 3 333 346 5 149 184 7 247 247	-10 170 181 -11 161 -153 -12 285 284	* 111 -04 -1 580 -502 -2 150 -103 -3 02 -07	1 93 -86 -2 99 -73 -3 360 -369 -5 192 -190	• •	-6 261 260 -8 99 23 -9 101 63	1 284 271 -1 165 140 -2 109 -65 -5 128 -102
5 791 -757 6 621 600 8 306 311 -1 177 -181	3 1322 -1389 4 461 -466 5 382 -586 6 228 225	3 116 -93 4 287 -277 6 395 -385 8 269 -267	-1 753 803 -2 113 116 -3 911 885 -6 85 -82	2 13	-1 293 304 -2 340 -347 -4 467 -467 -5 78 -37	4 4 0 149 -179	-5 542 -531 -6 623 -619 -7 117 -102 -6 521 -516	-7 359 -343 -8 150 -123 -11 254 -259	-6 279 -286 -8 254 -257 -10 276 -287	1 112 93 -1 141 119	-7 202 -194 -9 253 -260 -11 309 -304 -13 275 -274
-2 522 -532 -3 257 240 -5 796 -757 -6 624 -600	7 559 -533 8 123 -99 9 223 -215 10 110 84	10 204 -203 -1 121 -130 -2 120 110 -3 130 -127	-5 555 523 -6 206 -196 -7 309 301 -8 512 -503	-1 173 106 -5 97 53 -3 0	-6 291 -290 -7 177 -155 -8 272 -269 -9 231 -233	2 146 -133 3 176 -165 6 379 375 8 277 279	-9 186 182 -10 125 -112 -11 158 158 -12 219 -210		0 220 210 1 129 44	-6 254 249 -8 149 142 -10 158 166	9 100 92 9 100 92
10 379 570 12 121 104 -10 379 -370 -13 108 -104	12 104 128 -1 245 234 -2 247 249 -3 222 -212	-6 265 279 -8 278 276 -10 257 256 -12 156 156	-10 302 -317 -12 262 -275 -14 161 -160	7 1444 1740 4 523 555 4 401 405		-3 200 134 -2 646 -673 -3 255 -240 -4 105 -159	3 2	-2 193 -162 -3 252 252 -6 252 -522 -6 152 -159	2 175 146 -2 299 317 -3 173 -166 -9 211 711	7 10 -3 112 113 -7 300 289	-1 145 123 -3 257 243 -4 130 127 -5 153 152
0 e 0 125 1ee	-4 877 -844 -5 562 533 -6 359 -344 -7 690 880	1 9	2 4 0 136 148 1 222 310	-2 80 84 -4 126 -119 -8 1253 -1213 -10 460 -468	0 218 252 2 448 470 4 347 373 6 322 323	-6 775 -773 -8 618 -597 -10 342 -538 -12 104 -118	2 353+ 51 3 394+ 107 3 253 254 4 131 114	5 12 0 223 222 -4 150 110	-5 135 -138 -6 109 112 -7 206 -206 -9 187 -193	-4 123 134 8 0	-6 139 -132 -7 259 241 -8 99 -110 -9 116 128
1 261 266 2 200 -191 3 670 665 4 338 309	-9 477 478 -10 243 237 -11 290 800	2 282 -289 3 333 -334 4 100 -109 9 320 -114	7 355 578 3 187 -185 4 71 51 7 139 128	-14 375 -105	-1 146 136 -3 122 106 -4 107 -101	• •	7 242 203 9 269 256 -1 269 -275 -2 77 67	-4 149 130	6 10 0 99 -32	2 198 -189 6 220 -190 -4 112 116 -6 245 245	-10 140 -134 -12 143 -148
6 128 -124 7 497 503 9 249 246 -1 242 -266	-14 LOB L20	6 98 -82 7 154 -151 8 147 145 9 153 -148	11 117 -89 -1 186 -186 -2 1217 1224 -3 80 68	0 642 644 1 341 362 2 78 -64 3 666 661	-6 205 -218 -7 126 117 -8 230 -238 -10 264 -270	2 512 512 3 269 -267 6 361 367 6 195 195	-4 114 99 -5 746 -758 -6 126 124 -7 424 -616	-10 88 -56	1 226 228 -1 246 244 -2 119 115 -3 228 211	-8 284 268 -10 409 415 -12 270 281	0 158 132 -2 236 236 -3 236 -140 -4 457 436
-2 192 -191 -3 666 -665 -6 330 309 -5 599 -592	0 136 134 1 1173 -1317 2 263 267 3 876 -408	-1 95 89 -2 531 -497 -3 80 -61 -4 509 -448	-4 1239 1105 -5 67 -35 -6 849 810 -8 278 271	6 262 247 5 180 178 7 257 267 6 126 -127	-12 217 -215	7 233 231 9 152 153 -1 253 -270 -2 427 443	-1 122 -132	-4 322 -320 -6 540 -526 -8 503 -501 -12 736 -765	-7 46 49 -9 113 -127 -10 152 193	0 349 -326 1 164 -139 2 102 -108	-5 200 193 -6 93 78 -8 359 348 -10 183 176
-7 508 -503 -9 272 -266 11 425 435 -11 436 -435	5 256 -261 9 155 166 10 82 87 11 271 269	-+ 1+7 -174 200 241 -+ 274 214	-10 130 120 -10 130 120 -10 110 -103 -2 5	10 149 -146 11 99 -92 -1 1099 1159 -2 830 839	1 233 753 2 164 140 3 162 161 4 156 -179	-5 315 -299 -6 91 97 -7 287 -282 -8 251 -251	1 341 375 1 341 375 3 31 -53 3 272 340		6 11 -2 103 69 -7 256 233	3 133 -108 4 109 -01 -1 194 -179 -2 296 -286	9 5 0 136 -130 -1 173 167
0 7	13 247 231 -1 741 -724 -2 49 46 -3 846 -767	1 10 0 237 -265 1 395 -407	1 984 1045 2 620 -635 3 137 151	-3 448 433 -4 831 766 -3 136 -132 -6 82 72	5 152 159 7 98 79 8 106 -136 -1 269 259	-9 187 -178 -10 330 -319 -11 127 158 -12 131 -118	4 249 297 5 173 165 6 149 157 8 219 165	2 203 276 3 248 251 4 320 324 5 240 225	7 0 9 302 314	-3 100 -85 -4 390 -388 -6 245 -260 -7 276 273	-3 218 217 -5 247 245 -7 113 107 -10 206 197
2 172 148 3 344 351 4 367 346 5 182 184 6 130 334		3 187 -180 4 173 183 5 273 -267 7 93 114	• 374 -393 • 104 111 • 520 -520 7 147 136 • 230 -340		-2 318 326 -3 244 257 -4 409 400 -3 137 -134 -6 378 137	-16 223 -267	-1 348 373 -2 227 -233 -3 429 447 -4 386 -386	+ 109 + + + + + + + + + + + + + + + + + + +	-2 557 550 -6 472 406 -6 812 793 -12 213 -243	-10 11C -110 -11 216 230 -13 175 136	-11 95 -17 -12 127 119 9 6
7 176 180 8 289 278 -1 341 344 -2 167 -168	-13 135 149	-1 355 -342 -3 443 -404 -4 244 271 -5 274 -255	10 147 -150 11 203 -205 -1 74 84 -2 369 -373	-14 102 -148	-8 535 517 -9 167 -157 -11 156 -169	1 334 372 2 156 161 3 784 832 5 121 121	-7 188 185 -8 475 -480 -9 188 -185	-5 405 -400 -6 264 272 -7 481 -469	7 L 1 333 330	8 2 3 396 -336	-4 123 113 -6 213 -204 -9 298 297 -11 207 303
-3 361 351 -4 365 -366 -5 177 184 -6 325 -324	0 798 -920 1 882 919 2 608 -608 3 841 843 4 184	-6 145 -145 -7 117 -114 -8 152 -157 -9 156 -129	-5 281 283 -6 209 202 -5 178 176 -6 492 459	0 242 -250 1 455 496 2 80 -80 3 117 111	3 10 0 140 -147 1 338 346	+ 121 -129 7 152 185 -1 401 523 -2 125 -135	-10 262 -274 -11 146 -136 -12 184 -210 -13 267 -272	-9 308 -305 -10 197 -211 -11 406 -403 -12 274 -284	5 105 -160 3 113 46 4 302 -269 5 128 129 9 198 -144	3 115 -101 -1 264 -253 -3 472 -457 -4 125 106	+ 7
-7 170 100 -0 205 -270 10 199 215 11 116 -121 -10 222 -213	5 824 813 7 407 410 8 84 73 9 292 302	1 11 0 134 -349 1 195 188	-0 116 136 -0 400 402 -10 424 402 -11 104 -101	6 40 -61 7 259 -257 9 305 -299 11 189 -175	-1 203 208 -2 179 -175 -3 439 433 -4 206 205	-3 269 273 -4 344 345 -5 309 312 -7 265 -259	3 4	-13 108 -65 -14 102 -65	-1 296 291 -2 99 117 -3 143 145 -6 276 276	-7 226 -232	-5 240 -234 -8 132 125 -9 129 -122
-11 100 -121 -12 110 -95	10 87 102	3 146 132	-12 183 185	-1 057 1022	-5 223 200	-9 345 -345	2 294 108	0 173 -170	-5 391 386 -6 212 209 -7 93 -91	0 154 -123 1 117 113	-6 190 -170 -8 180 -153

# Table 3. Structure factors

Each group of three columns contains l,  $10|F_o|$  and  $10F_c$ , and is headed by the values of h and k common to the group. Asterisks following  $10|F_o|$  mark reflections omitted from refinements.





Fig. 1. (a) Interatomic distances. The standard deviation is about 0.007 Å for bonds between heavy atoms and 0.05 Å for bonds involving hydrogen. (b) Bond angle standard deviations are about 0.8° for angles involving only heavy atoms, and 4° for those involving hydrogen atoms. H(27)-C(16)-H(28) is 114°, H(28)-C(16)-H(29) is 112°, and H(28)-C(16)-N(14) is 112°.

factors gave R = 5.6%. Isotropic refinement of the hydrogen atoms then produced an R of 5.3%. The remaining two water hydrogen atoms were located on a difference map, the imaginary component of the bromine anomalous scattering factor was included in refinement, and a final R of 4.8% was obtained.

Scattering factors were those in International Tables for X-ray Crystallography (1962) except for hydrogen (Stewart, Davidson & Simpson, 1965). Computations were performed on the Tulane Computer Laboratory IBM 7044 computer with programs: BLSA for leastsquares, modified from the block-diagonal form of UCLALS 1 (Gantzel, Sparks & Trueblood, 1962); FOUR for Fourier summation (C.J.Fritchie, Jr); GSET4 for goniometer orientation (C.T.Prewitt); ORTEP for plotting figures (C.Johnson); and CELL for determining unit-cell constants (B.L.Trus).

The final positional parameters appear in Table 1 and the thermal parameters in Table 2. Observed structure factors are given in Table 3. Of the unobserved structure factors, 40 exceed the observational threshold by amounts less than  $\sigma_F$ , twelve have errors in the range  $\sigma - 2\sigma$ , one in the range  $2\sigma - 3\sigma$ , and two have larger relative errors. These latter (102 with  $F_0 \le 4.3$ ,  $F_c = -10.1$  and  $\sigma_F = 0.90$ ; and 020 with  $F_0 \le 3.8$ ,  $F_c =$ -13.8 and  $\sigma_F = 1.03$ ) are believed to have standard deviations, and probably minimum observable magnitudes, which are seriously underestimated.

# Discussion

# Molecular configuration

Dudley *et al.* (Dudley, Ehrenberg, Hemmerich & Müller, 1964) have deduced, on spectroscopic grounds, that protonation of isoalloxazine (flavin) occurs at N[1]. N[1] is in fact the site of protonation in riboflavin hydrobromide hydrate (Tanaka, Ashida, Sasada & Kakudo, 1967, hereafter TASK), and also in the present structure. Both of these structures also confirm that the generally accepted diketo tautomer is the correct one.

The bond lengths given by TASK agree with ours [Fig. 1(a)] within  $2\sigma$  (TASK  $\sigma_{C-C} = 0.02$  Å) except for C(10)–C(11), for which they report a length of 1.47 Å. Even this is only  $2.5\sigma$  from our value of 1.415 Å. Their angles also agree with ours within  $3\sigma$ . Our values appear in Fig. 1(b). Surprisingly, bond lengths in 1.3.10-trimethylisoalloxazinium iodide (Kierkegaard, Norrestam, Werner, Ehrenberg, Eriksson & Müller, 1967) differ by 0.04-0.10 Å from ours in several instances, despite their low R value (5.4%,  $\sigma$  unstated). The disagreement is probably a result of the heavy iodine atom in the trimethylisoalloxazinium salt. Supported by the work of TASK, we consider our parameters accurate to within the standard deviations given by the least-squares analysis ( $\sigma_{C-C} =$ 0.007 Å and  $\sigma_{C-C-C}=0.8^{\circ}$ ). The bond lengths in the CO-NH-CO region of the molecule are in good agreement with values in several similar molecules, as is shown in Table 4. Similarly, the generally large intracyclic angles at nitrogen atoms in the outer ring are a common feature of such systems (Singh, 1965*a*, 1965*b*; Reeke & Marsh, 1966).

# Table 4. Comparison with bond lengths in similar molecules

Bond C(3)-O(17)	This work 1∙209 Å	RM 1∙229 Å	S 1∙208 Å
C(3)-N(4)	1.379	1.363	1·209 1·370 1·379
N(4)-C(5)	1.388	1.381	1.374
C(5)-O(18)	1.211	1.230	1.221

RM is 5-ethyl-6-methyluracil, Reeke & Marsh (1966). ( $\sigma_{bond} = 0.005 \text{ Å}$ ); S is alloxan (hydrated), Singh (1965a). ( $\sigma_{bond} = 0.005 \text{ Å}$ ).

Isoalloxazine bond lengths, calculated from the results of Pariser-Parr-Pople self-consistent-field molecular orbital calculations (Fox, Nishimoto & Forster, 1965) by use of the Nishimoto-Forster (Nishimoto & Forster, 1966) approximations, are compared with the observed values in Table 5. The agreement in the phenylene ring is particularly good. The prediction that C(9)-C(10) and C(11)-C(12) are shorter and other bonds longer than the benzenoid value of 1.396 Å is verified. Comparison beyond this point becomes uncertain since the calculations were performed for neutral, rather than N[1] protonated, flavin. However, bonds N(7)-C(8) and C(13)-N(14) are expected to change but little upon protonation, as is the CO-NH-CO grouping. The observed and calculated values here are in reasonable agreement. considering the predilection of the method for predicting long C-O bonds (Fox, Laberge, Nishimoto & Forster, 1967).

# Table 5. Comparison of calculated with observed distances

Bond	Calculated*	Observed
N(2) - C(3)	1·379 Å	1·408 Å
C(3) = O(17)	1.271	1.209
C(3) - N(4)	1.379	1.379
N(4) - C(5)	1.366	1.388
C(5) - O(18)	1.268	1.211
C(5) - C(6)	1.466	1.489
C(6) - N(7)	1.308	1.299
N(7) - C(8)	1.368	1.376
C(8) - C(9)	1.415	1.411
C(9) - C(10)	1.387	1.348
C(10) - C(11)	1.407	1.415
C(11) - C(12)	1.389	1.362
C(12) - C(13)	1.411	1.419
C(13) - N(14)	1.379	1.386
N(14) - C(15)	1.363	1.338
C(15) - N(2)	1.323	1.364
C(15) - C(6)	1.450	1.422
C(13) - C(8)	1.414	1.407

\* Fox, Nishimoto & Forster, 1965. Comparison is not exact, because the calculation was performed for the non-protonated species (see text).

It is interesting that the quasi double nature of C(6)–N(7) and quasi-single natures of C(5)–C(6) and N(2)–C(3) which are predicted by the simplest resonance forms are in fact found. The fact that N(14)–C(15) is shorter than N(2)–C(15) probably implies a greater (positive) charge at N(14), a prediction in accord with the general behavior of carbonium ions.

The molecule is nearly planar, as shown by the leastsquares plane results in Table 6. It is, however, bowed to a significant degree along its long axis. Similar calculations of planes through each of the three rings separately showed that the distortion is fairly uniform over the molecule, the average deviation from each of the planes being approximately 0.01 Å. The methyl carbon atom shows no larger deviation from the plane of the central ring than do the ring atoms themselves. The two oxygen atoms are somewhat further from the least-squares plane of the end ring, about 0.03 Å.

## Packing

Because of the interest in understanding the flavinenzyme and possible flavin-substrate binding in the course of flavo-protein redox reactions and the difficulty in obtaining simple metal complexes of flavins (Hemmerich, Müller & Ehrenberg, 1965), it is important to make whatever deductions possible regarding the relative basicities of the various nitrogen and oxygen atoms. Fortunately rather extensive hydrogen bonding exists in this crystal, and some comparisons are thus possible. At the same time, any deductions made on the basis of hydrogen bonding schemes must be treated with care, since under various conditions differing schemes may prevail (Hoogsteen, 1963; Haschemeyer & Sobell, 1965).

The part of the hydrogen bonding system relevant to a discussion of the properties of 10-methylisoalloxazine is given in Fig. 1(a) and (b). Atoms O(19) and

#### Table 6. Least-squares plane

	Deviation		Deviation
N(2)	0·012 Å	N(14)	−0•082 Å
C(3)	0.044	C(15)	-0.042
N(4)	0.069	C(16)	-0.087
C(5)	-0.016	O(17)	0.057
C(6)	-0.039	O(18)	-0.032
N(7)	-0.042	H(21)	0.006
C(8)	-0.030	H(22)	-0.042
C(9)	-0.004	H(23)	-0.018
C(10)	0.057	H(24)	0.028
C(11)	0.108	H(25)	0.178
C(12)	0.035	H(26)	0.069
CÌLISÍ	-0.029		

The plane is given by the equation 0.68260m - 0.01943n - 0.73053p = -1.8435, where *m*, *n* and *p* are parameters measured along orthonormal axes  $\mathbf{m} || \mathbf{b} \times \mathbf{c}^*$ ,  $\mathbf{n} || \mathbf{b}$  and  $\mathbf{p} || \mathbf{c}^*$ . Only non-hydrogen atoms were used in calculating the plane, and each was given its atomic number as weight. The method of Blow (1960) was used in calculating the plane.

Br(1) serve as receptors for hydrogen bonds from N(2)-H(21) and N(4)-H(22) respectively. The N-H···O bond is characterized by  $H(21) \cdots O(19) = 2.14 \text{ Å}$ ,  $N(2) \cdots O(19) = 2.90$  Å, and  $< NHO = 159^{\circ}$ . The more reliable heavy atom separation is intermediate in the range of about 2.87-3.04 Å given in a recent tabulation by Hamilton & Ibers (1968). In the case of the  $NH \cdots Br$  bond,  $N(4) \cdots Br(1)$  is 3.44 Å,  $H(22) \cdots Br(1)$ is 2.42 Å, and < NHBr is 169°. The H...Br distance is 0.7 Å, smaller than the sum of van der Waals radii (3.15 Å; Pauling, 1948) and must thus be considered a bond. The  $N \cdot \cdot \cdot Br$  distance of 3.44 Å is only slightly longer than distances proposed as hydrogen bonds in a number of recent structures (3.36, 3.32 Å, Subramanian, 1967; 3.35 Å, Kadoya, Hanazaki & Iitaka, 1966; 3.40 Å (bifurcated), Przybylska, 1965).

Of the three possible hydrogen-bond receptor sites, O(17), O(18), and N(7), only O(18) actually partici-



Fig. 2. A portion of the hydrogen bonding system.  $O(19'') \cdots O(20'')$  is 2.98 Å,  $O(18'') \cdots O(19''')$  is 2.89 Å, and  $O(19'') \cdots O(20)$  is 2.78 Å.

pates in bonding, to H(31') of a water molecule,  $O(18) \cdots H(31')$  is 2.16 Å,  $O(18) \cdots O(19')$  is 2.89 Å. and angle OHO is 152°. The same H(31') approaches

N(7) within 2.55 Å. This distance, only 0.15 Å less than the sum of van der Waals radii (Pauling, 1948) is not considered to be a hydrogen bond but may be



Fig. 3. [010] projection. Some of the hydrogen bonds shown are to molecules in front of, or behind, those pictured.



Fig.4. [001] projection. Some of the hydrogen bonds shown involve molecules in front of, or behind, those pictured.

a stabilizing van der Waals contact. The orientation of the water molecule H(30')-O(19')-H(31') is probably determined chiefly by a hydrogen bond to O(20), shown in Fig. 2. Thus what first appears as an attempt by the structure to form a bifurcated bond from H(31')to both O(18) and N(7) is probably artefactual. We can conclude, with the reservations given above, that O(18) is the most basic of the three receptor atoms in protonated flavin.

The remainder of the hydrogen-bonding system is shown in Fig.2 and two projections, Figs.3 and 4. Water molecule H(32)-O(20)-H(33) is not bound to flavin, but to two other water molecules and to the bromide ion. O(19) is in a roughly tetrahedral environment, serving as a double hydrogen-bond donor and as a double acceptor. O(20) is a double donor and single receptor, and is in a pyramidal environment.

As Fig.3 illustrates, all components of the structure lie in layers more or less parallel with (204). These layers contain some of the hydrogen bonds, with hydrogen bonds between water molecules serving to link the layers pairwise. Fig.4 illustrates more clearly the intralayer hydrogen bonding. Flavin molecules within a layer are linked throughout by hydrogen bonds involving both the bromide ion and water molecules.

There is little direct interaction between flavin molecules, as can be seen in Fig.4. One layer is shown in a fairly complete fashion with a single flavin molecule above and one below this sheet. The upper molecule exhibits flavin-flavin contact through C(10)-O(18) and H(23)-N(4). The only contact with the lower molecule is H(23)-N(7). The bromide ion, in addition to being a double hydrogen bond acceptor, lies fairly close to the presumably most positive areas of the flavin molecules above and below it. Thus, it is near N(14) of the upper molecule and N(2) of the lower molecule.

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