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The Crystal and Molecular Structure of Two Models of Catalytic Flavo(co)enzyme Intermediates

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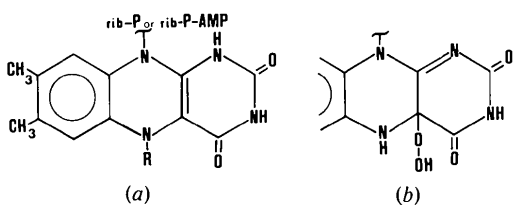
Two stable derivatives of 'reduced' 4a,5-dihydroflavin serving as models for enzymic catalytic intermediates have been synthesized and crystallized: 4a-isopropyl-3-methyl-4a,5-dihydrolumiflavin ($C_{17}H_{22}N_4O_2$) (I) and 4a,5-epoxyethano-3-methyl-4a,5-dihydrolumiflavin ($C_{16}H_{18}N_4O_3$) (IV); (I) belongs to space group $C2/c$, Z = 8, with unit-cell dimensions a = 11·757 (2), b = 14·060 (2), c = 19·785 (2) Å, β = 91·38 (2)°; (IV) is monoclinic, space group $P2_1/n$, Z = 4, a = 12·665 (2), b = 15·217 (2), c = 7·804 (2) Å, β = 95·66 (2)°. Both structures have been solved by means of direct methods and refined by full-matrix least squares to final weighted R values of 0·046 for (I) and 0·041 for (IV). In contrast to the non-planar 1,5-dihydroflavins these 4a,5-dihydroflavins are almost planar, the dihedral angle measured along the N(5)—N(10) axis being 3·2° in the first case and 4·9° in the second. Ordinary van der Waals contacts are found in (IV), while in (I) two hydrogen bonds link two molecules to one another. Data for two further flavin derivatives [compounds (II) and (III)] have been collected.

Introduction

The isoalloxazine moiety is the redox-active part of riboflavin (vitamin B_2); its FMN and FAD forms* constitute the coenzymes of the ubiquitous flavin enzymes. The molecular mechanism by which these enzymes catalyze most different redox processes, *e.g.* electron transfer, C—H oxidation, oxygen activation, has been the object of extensive investigation in recent years [see several articles in *Flavins and Flavoproteins* (1976)]. In particular, it has been proposed that oxidation of α-OH and α-NH_2 carboxylic acids occurs through transient formation of covalent N(5) adducts to the reduced (1,5-dihydro)flavocoenzymes (Porter, Voet & Bright, 1973; Massey & Ghisla, 1975) (Fig. 1*a*).

* FMN and FAD stand for flavin mononucleotide and flavin adenine dinucleotide.



(*a*) (*b*)

Fig. 1. Schematic formulae of FMN or FAD adducts. R = oxidized substrate.

The structures of a series of substituted 1,5-dihydroflavins, which are stable to oxygen, were investigated by X-ray techniques and found to be bent along the N(5)—N(10) axis (Kierkegaard *et al.*, 1971). Activation of molecular oxygen in flavoenzyme-catalyzed hydroxylation reactions has recently been suggested to occur through C(4a) peroxide adducts to the 4a,5-dihydroflavocoenzymes (Entsch, Ballou & Massey, 1976; Ghisla, Entsch, Massey & Husein, 1977).

The intermediates responsible for hydroxylation were characterized by stopped-flow kinetics (Entsch *et al.*, 1976), and their structures deduced from comparison of the observed transient intermediates with enzyme-bound 4a,5-dihydroflavin derivatives of FAD (Ghisla *et al.*, 1977). Kemal & Bruice (1976) recently reported the isolation of a stable N(5)-blocked 4a-peroxide derivative of 4a,5-dihydroflavin [compare with Fig. 1(*b*)], and pointed out its spectral similarity to the (metastable) oxygenated bacterial luciferase characterized by Hastings, Ballny, Le Peuch & Douzou (1973). In general, the electronic spectra of reduced flavins, free in solution or enzyme-bound, were found to be strongly affected by temperature, molecular geom-

etry and environment, *i.e.* solvent, protein, presence of bound substrates or effectors (Ghisla, Massey, Lhoste & Mayhew, 1974; Entsch *et al.*, 1976; Ghisla *et al.*, 1977).

These observations prompted us to provide further information about the structure of reduced-flavin models analogous to Fig. 1(*a*) and (*b*) with the aim of obtaining a correlation between structure and spectral properties of models on the one hand and thus the basis for comparisons with the catalytic enzyme intermediates on the other. Furthermore, knowledge of the structural parameters around the redox-active moiety C(4a)–N(5) might help in the clarification of the peculiar chemistry of reduced flavins (Ghisla, Hartmann, Hemmerich & Müller, 1973) and the mechanism of oxygen activation in flavin 4a-peroxides.

The present report deals with two derivatives of 4a,5-dihydroflavin [compare with Fig. 1(*b*)]. The first model [Fig. 2 (I)] was chosen because it would represent the first oxygen-stable C(4a)-monosubstituted derivative of a 4a,5-dihydroflavin. The 4a,5-bridged model [Fig. 2 (IV)] was chosen as the analog best simulating the spectral properties of catalytic intermediates observed with *p*-hydroxybenzoate hydroxylase (Ghisla *et al.*, 1977); the rigidity introduced by the 4a–5 bridge into the model might simulate a strain induced by the protein environment on the protein-bound intermediate. The crystal structure of a 4a,5-dihydroflavin blocked at positions C(4a) and N(5), which had been crystallized by one of us, has been reported recently (Norrestam, 1972).

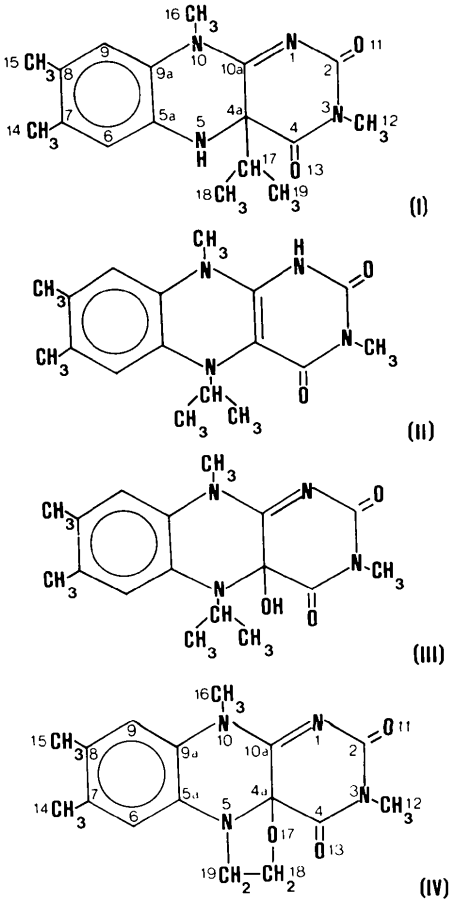


Fig. 2. Formulae and atomic numbering of the compounds studied. (I) 4a-Isopropyl-3-methyl-4a,5-dihydrolumiflavin. (II) 5-Isopropyl-3-methyl-1,5-dihydrolumiflavin. (III) 4a-Hydroxy-5-isopropyl-3-methyl-4a,5-dihydrolumiflavin. (IV) 4a,5-Epoxyethano-3-methyl-4a,5-dihydrolumiflavin.

Experimental data

Synthesis and crystallization of the compounds

4a-*Isopropyl*-3-*methyl*-4a,5-*dihydrolumiflavin* ($C_{17}H_{22}N_4O_2$) (I) *and* 5-*isopropyl*-3-*methyl*-1,5-*dihydrolumiflavin* (II) ($C_{17}H_{22}N_4O_2$) were synthesized as described earlier (Ghisla *et al.*, 1973). Crystals of (I) were obtained by addition of small amounts of water to a saturated methanol solution at room temperature. (II) was crystallized by slow cooling from a saturated chloroform/cyclohexane solution. Both compounds were crystallized as soon as possible after their purification in order to avoid contamination with oxidation products.

4a-*Hydroxy*-5-*isopropyl*-3-*methyl*-4a,5-*dihydrolumiflavin* ($C_{17}H_{22}N_4O_3$) (III) was obtained as yellow-orange prisms by addition of a slight excess of solid sodium nitrite to a saturated solution of (II) in 0·1 *N* acetic acid and subsequent cooling. The product obtained by this procedure was identical with that described earlier (Ghisla *et al.*, 1973).

4a,5-*Epoxyethano*-3-*methyl*-4a,5-*dihydrolumiflavin* (5,8,10,11-*tetramethyl*-1,2-*dihydro*-8*H*-*benz*[*g*]*oxazolo*-[2,3-*e*]*pteridin*-4,6-*dione*) ($C_{16}H_{18}N_4O_3$) (IV) was synthesized by adaptation of the reductive alkylation procedure described earlier (Ghisla *et al.*, 1973). 3 g of 3-methyllumiflavin were suspended in 200 ml of dimethylformamide with addition of sodium dithionite (3·5 g) and potassium carbonate (7·0 g) under a nitrogen stream. The suspension was stirred at 50°C, 7·0 ml of 2-iodoethanol were then added and the course of alkylation was followed by thin-layer chromatography on silica-gel plates (butanol:acetic acid:water = 4:3:1, detection by fluorescence). After 3 h an additional aliquot of 2-iodoethanol was added. The reaction was stopped when most starting material had disappeared (~60 min) by adjusting the pH of the mixture to 5·0 with acetic acid. Dimethylformamide evaporated under vacuum, the precipitate was dissolved in chloroform and extracted with 0·1 *N* ammonia/ethanol (9:1), and sodium dithionite was added when

necessary during the extraction in order to keep the mixture just reduced. The resulting solution turned black upon admission of oxygen and was left overnight in an open vessel. The stable blue radical of 5-β-hydroxyethyl-3-methyl-1,5-dihydrolumiflavin, crystallized in small needles, was separated by filtration and dried *in vacuo*. (IV) was formed directly by oxidation of the blue radical with NO_2^-. Small aliquots were dissolved in a minimum amount of acetonitrile and a solution of sodium nitrite in 0·1 *N* acetic acid was added until complete disappearance of the blue color and appearance of a yellow-orange solution. Excess of NO_2^- enhances the decay of (IV). The product crystallized from this solution upon cooling. Some of its physical data have been described elsewhere (Ghisla *et al.*, 1977).

X-ray data collection

(I), (II), (III) and (IV) were examined by means of a Philips PW 1100 four-circle diffractometer with graphite-monochromatized radiation. (I) was found to be monoclinic, space group $C2/c$ from systematic absences and structure analysis, $a = 11{\cdot}757\ (2)$, $b = 14{\cdot}060\ (2)$, $c = 19{\cdot}785\ (2)$ Å, $\beta = 91{\cdot}38\ (2)°$, $Z = 8$. The other monoclinic compound was (IV): space group $P2_1/n$ from systematic absences, $a = 12{\cdot}665\ (2)$, $b = 15{\cdot}217\ (2)$, $c = 7{\cdot}804\ (2)$ Å, $\beta = 95{\cdot}66\ (2)°$, $Z = 4$. (II) and (III) were triclinic; their lattice parameters are given in Table 1. Space group $P\bar{1}$ was assumed for both of them on the basis of the statistical averages and distribution of the normalized structure factors. The X-ray diffraction data of (I) were collected with Mo $K\alpha$ radiation (θ less than 21°) and the ω–2θ scan technique; three standard reflections monitored at 3 h intervals showed no variation greater than 3·8% in intensity. Processing of the data was carried out in the manner described by Davies & Gatehouse (1973) to yield the values of F_o and σF_o. Of the 1759 measured reflections, 1292 were found to have $I > 2\sigma(I)$ and were used in the subsequent calculations. In the case of (IV) Cu $K\alpha$ radiation was used to collect 2741 reflections (θ range 3–68°; ω–2θ scan mode); the maximum deviation of the standard reflections was 5%. The X-ray data were processed as for (I); 1255 reflections had $I > 3\sigma(I)$ and were considered as observed.

Table 1. *X-ray crystal data of compounds* (II) *and* (III)

(II)	(III)
$C_{17}H_{22}N_4O_2$	$C_{17}H_{22}N_4O_3$
Space group $P\bar{1}$	Space group $P\bar{1}$
$Z = 2$	$Z = 2$
$a = 9{\cdot}721\ (6)$ Å	$a = 11{\cdot}008\ (3)$ Å
$b = 11{\cdot}417\ (8)$	$b = 11{\cdot}494\ (4)$
$c = 9{\cdot}631\ (5)$	$c = 9{\cdot}244\ (2)$
$\alpha = 104{\cdot}39\ (3)°$	$\alpha = 106{\cdot}83\ (2)°$
$\beta = 90{\cdot}74\ (3)$	$\beta = 110{\cdot}03\ (2)$
$\gamma = 82{\cdot}81\ (3)$	$\gamma = 102{\cdot}47\ (2)$
2789 collected reflections	1201 collected reflections

Structure solution and refinement

(*a*) 4a-Isopropyl-3-methyl-4a,5-dihydrolumiflavin (I) was solved in a straightforward manner by use of direct methods with *MULTAN* (Germain, Main & Woolfson, 1971). The first *E* map revealed 19 atoms; the *R* factor estimated at this level was 0·49. A subsequent electron density map showed all 23 non-hydrogen atoms and *R* dropped to 0·36.

After isotropic convergence, three cycles of full-matrix least-squares anisotropic refinement were carried out and all H atoms were located on a difference electron density map. The C–H bond distances ranged from 0·87 to 1·09 Å and bond angles involving H atoms were in the range of expected values. In the last two cycles, coordinates and isotropic temperature factors of the H atoms were refined too. Convergence was considered attained when the atomic shifts were less than one e.s.d. The final weighted R $\{R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}$ where $w = 1/\sigma F_o\}$ was 0·046 for the 1292 reflections with $I > 2\sigma(I)$.

(*b*) 4a,5-Epoxyethano-3-methyl-4a,5-dihydrolumiflavin (IV) was solved without any particular difficulty. Direct methods showed the full skeleton of the molecule with the exception of the epoxyethano bridge linked to N(5) and C(4a).

After isotropic least-squares refinement of the partial structure, the positions of O(17), C(18) and C(19) were determined from an electron density map. After anisotropic full-matrix least-squares refinement, all but five H atoms could be located from a difference electron density map; those missing, H(106),* H(118), H(218), H(119) and H(219), were given estimated positions. The C–H bond distances (from 0·86 to 1·11 Å) and the corresponding angles are within the limits of acceptable values.

In the final cycle, the non-hydrogen atoms were refined anisotropically together with the coordinates and isotropic temperature factors of the H atoms; three of them showed negative temperature factors, and were set equal to zero. The final R_w factor was 0·041 for the 1255 reflections with $I > 3\sigma(I)$. Atomic coordinates are listed in Tables 2 and 3,† bond lengths and angles in Tables 4 and 5. The atomic scattering factors used for

* H(106) means 'hydrogen 1 on carbon 6' *etc.*

† Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33072 (33 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 2. *Fractional atomic coordinates* ($\times 10^4$; *for* H $\times 10^3$), *with standard deviations, and isotropic temperature factors for hydrogen atoms, for compound* (I)

The mean standard deviation for thermal parameters is 0·5 Å².

	x	*y*	*z*		*x*	*y*	*z*	*B* (Å²)
N(1)	3388 (2)	146 (2)	3138 (1)	H(105)	361 (2)	192 (1)	489 (1)	4·5
C(2)	2279 (3)	376 (2)	3001 (2)	H(106)	525 (2)	218 (1)	557 (1)	3·8
N(3)	1696 (2)	979 (2)	3456 (1)	H(109)	719 (2)	14 (1)	413 (1)	3·4
C(4)	2245 (2)	1514 (2)	3933 (1)	H(112)	13 (2)	106 (1)	388 (1)	6·6
C(4a)	3538 (2)	1510 (2)	3929 (1)	H(212)	17 (2)	59 (1)	308 (1)	6·7
N(5)	3924 (2)	1527 (2)	4629 (1)	H(312)	25 (2)	169 (1)	317 (1)	6·7
C(5a)	5078 (2)	1373 (2)	4756 (1)	H(114)	695 (2)	239 (1)	627 (1)	6·5
C(6)	5679 (2)	1767 (2)	5295 (1)	H(214)	809 (2)	228 (1)	597 (1)	5·8
C(7)	6808 (3)	1551 (2)	5433 (1)	H(314)	759 (2)	150 (1)	640 (1)	6·4
C(8)	7375 (3)	941 (2)	4998 (2)	H(115)	913 (2)	119 (1)	508 (1)	6·9
C(9)	6795 (3)	559 (2)	4441 (3)	H(215)	880 (2)	50 (1)	556 (1)	6·6
C(9a)	5654 (2)	764 (2)	4316 (1)	H(315)	891 (2)	14 (1)	476 (1)	8·2
N(10)	5020 (2)	321 (2)	3780 (1)	H(116)	494 (2)	−85 (1)	320 (1)	5·2
C(10a)	3960 (3)	609 (2)	3605 (1)	H(216)	589 (2)	−96 (1)	380 (1)	5·4
O(11)	1750 (2)	47 (2)	2524 (1)	H(316)	613 (2)	−27 (1)	315 (1)	4·4
C(12)	452 (2)	1050 (3)	3397 (2)	H(117)	479 (2)	229 (1)	350 (1)	3·7
O(13)	1729 (2)	2013 (2)	4325 (1)	H(118)	403 (2)	381 (1)	362 (1)	5·3
C(14)	7406 (3)	1987 (2)	6038 (2)	H(218)	287 (2)	343 (1)	388 (1)	6·7
C(15)	8608 (3)	673 (2)	5109 (1)	H(318)	398 (2)	331 (1)	435 (1)	6·9
C(16)	5521 (3)	−505 (2)	3441 (1)	H(119)	272 (2)	256 (1)	278 (1)	4·9
C(17)	3967 (2)	2391 (2)	3510 (1)	H(219)	384 (2)	294 (1)	257 (1)	5·3
C(18)	3732 (3)	3323 (2)	3864 (2)	H(319)	378 (2)	178 (1)	248 (1)	8·6
C(19)	3516 (3)	2409 (2)	2783 (2)					

Table 3. *Fractional atomic coordinates* ($\times 10^4$; *for* H $\times 10^3$), *and isotropic temperature factors for hydrogen atoms, for compound* (IV)

The mean standard deviation for temperature factors is 1·2 Å².

	x	*y*	*z*		*x*	*y*	*z*	*B* (Å²)
N(1)	2117 (2)	4625 (2)	−1070 (3)	H(106)	−4 (3)	335 (2)	526 (4)	0·0
C(2)	2309 (3)	5525 (2)	−1011 (4)	H(109)	95 (3)	170 (2)	30 (4)	0·0
N(3)	1805 (2)	6043 (2)	139 (3)	H(112)	259 (3)	709 (2)	7 (4)	5·2
C(4)	1267 (3)	5715 (2)	1421 (4)	H(212)	153 (3)	726 (2)	87 (4)	5·1
C(4a)	1545 (2)	4729 (2)	1834 (4)	H(312)	156 (3)	716 (2)	−123 (4)	3·3
N(5)	841 (2)	4374 (1)	2968 (3)	H(114)	6 (3)	135 (2)	637 (5)	9·9
C(5a)	676 (2)	3441 (2)	2844 (4)	H(214)	−105 (3)	134 (2)	499 (4)	0·0
C(6)	166 (2)	3018 (2)	4100 (2)	H(314)	−79 (3)	208 (2)	631 (4)	3·7
C(7)	−23 (2)	2115 (2)	4065 (4)	H(115)	18 (3)	31 (2)	124 (4)	6·3
C(8)	278 (2)	1631 (2)	2664 (4)	H(215)	−63 (3)	53 (2)	267 (4)	3·2
C(9)	764 (3)	2058 (2)	1390 (4)	H(315)	54 (3)	52 (2)	350 (4)	3·4
C(9a)	972 (2)	2961 (2)	1458 (4)	H(116)	116 (3)	267 (2)	−196 (5)	6·7
N(10)	1467 (2)	3383 (2)	131 (3)	H(216)	220 (3)	231 (2)	−81 (4)	7·2
C(10a)	1687 (2)	4241 (2)	200 (4)	H(316)	208 (3)	327 (2)	−207 (4)	6·1
O(11)	2851 (2)	5872 (1)	−2018 (3)	H(118)	276 (4)	513 (4)	517 (7)	2·8
C(12)	1870 (4)	7015 (2)	−34 (6)	H(218)	282 (4)	412 (4)	494 (7)	8·8
O(13)	710 (2)	6141 (1)	2276 (3)	H(119)	112 (3)	431 (2)	581 (4)	6·0
C(14)	−512 (4)	1684 (3)	5548 (6)	H(219)	104 (3)	524 (2)	512 (4)	2·2
C(15)	83 (4)	642 (2)	2542 (7)					
C(16)	1731 (4)	2871 (3)	−1381 (5)					
O(17)	2605 (1)	4763 (1)	2783 (3)					
C(18)	2490 (3)	4675 (3)	4580 (4)					
C(19)	1299 (3)	4680 (2)	4704 (5)					

Table 4. *Bond lengths* (Å) *with standard deviations in parentheses, for* (I) *and* (IV)

	(I)	(IV)
N(1)–C(10a)	1·304 (4)	1·315 (4)
N(1)–C(2)	1·364 (4)	1·393 (4)
C(2)–N(3)	1·425 (4)	1·397 (3)
N(3)–C(12)	1·468 (4)	1·490 (4)
C(2)–O(11)	1·210 (4)	1·215 (3)
C(4)–N(3)	1·358 (4)	1·361 (3)
C(4)–O(13)	1·220 (4)	1·208 (3)
C(4)–C(4a)	1·522 (4)	1·570 (4)
C(4a)–N(5)	1·447 (3)	1·424 (3)
C(4a)–C(10a)	1·509 (4)	1·503 (4)
C(4a)–C(17)	1·580 (4)	
C(17)–C(19)	1·522 (4)	
C(17)–C(18)	1·513 (4)	
C(5a)–N(5)	1·388 (4)	1·439 (3)
C(5a)–C(6)	1·397 (4)	1·385 (4)
C(5a)–C(9a)	1·393 (4)	1·388 (4)
C(7)–C(6)	1·384 (4)	1·397 (4)
C(7)–C(14)	1·504 (4)	1·517 (4)
C(7)–C(8)	1·396 (4)	1·403 (4)
C(8)–C(9)	1·391 (5)	1·383 (4)
C(8)–C(15)	1·508 (4)	1·528 (4)
C(9)–C(9a)	1·389 (4)	1·402 (4)
N(10)–C(9a)	1·426 (4)	1·417 (3)
N(10)–C(10a)	1·349 (4)	1·337 (3)
N(10)–C(16)	1·470 (4)	1·481 (4)
O(17)–C(4a)		1·471 (3)
C(19)–N(5)		1·497 (4)
C(19)–C(18)		1·523 (5)
C(18)–O(17)		1·432 (3)

Table 5. *Bond angles* (°) *with standard deviations in parentheses, for* (I) *and* (IV)

	(I)		(IV)
C(2)–N(1)–C(10a)	119·8 (3)		119·9 (3)
N(1)–C(2)–N(3)	119·2 (3)		119·2 (3)
N(1)–C(2)–O(11)	122·4 (3)		121·0 (3)
N(3)–C(2)–O(11)	118·2 (3)		119·6 (3)
C(2)–N(3)–C(4)	122·7 (3)		124·0 (3)
C(2)–N(3)–C(12)	119·1 (3)		118·1 (3)
C(4)–N(3)–C(12)	118·2 (2)		117·9 (3)
N(3)–C(4)–O(13)	121·8 (3)		124·9 (3)
N(3)–C(4)–C(4a)	116·9 (2)		112·6 (3)
O(13)–C(4)–C(4a)	121·2 (3)		122·1 (3)
N(5)–C(4a)–C(4)	106·6 (2)		110·5 (2)
N(5)–C(4a)–C(17)	113·0 (2)	N(5)–C(4a)–O(17)	107·3 (2)
C(4)–C(4a)–C(17)	109·4 (2)	C(4)–C(4a)–O(17)	104·2 (2)
N(5)–C(4a)–C(10a)	108·7 (2)		118·3 (3)
C(4)–C(4a)–C(10a)	110·1 (2)		110·0 (3)
C(10a)–C(4a)–C(17)	108·9 (2)	C(10a)–C(4a)–O(17)	105·4 (2)
C(4a)–C(17)–C(19)	113·6 (2)		
C(4a)–C(17)–C(18)	111·9 (2)		
C(19)–C(17)–C(18)	111·1 (2)		
		C(4a)–N(5)–C(19)	103·5 (2)
		C(5a)–N(5)–C(19)	114·2 (3)
C(5a)–N(5)–C(4a)	116·1 (2)		115·4 (3)
N(5)–C(5a)–C(6)	122·8 (2)		119·1 (3)
N(5)–C(5a)–C(9a)	118·8 (2)		121·6 (3)
C(6)–C(5a)–C(9a)	118·3 (3)		119·3 (3)
C(5a)–C(6)–C(7)	122·2 (3)		122·3 (3)
C(6)–C(7)–C(14)	119·8 (3)		119·6 (3)
C(6)–C(7)–C(8)	118·9 (3)		118·3 (3)
C(8)–C(7)–C(14)	121·3 (3)		122·1 (3)
C(7)–C(8)–C(9)	119·6 (3)		119·3 (3)
C(9)–C(8)–C(15)	118·1 (3)		119·9 (4)
C(7)–C(8)–C(15)	122·4 (3)		120·8 (4)
C(9a)–C(9)–C(8)	121·0 (3)		122·0 (3)
N(10)–C(9a)–C(9)	121·8 (3)		120·8 (3)
N(10)–C(9a)–C(5a)	117·9 (3)		120·4 (4)
C(5a)–C(9a)–C(9)	120·1 (3)		118·8 (3)
C(9a)–N(10)–C(10a)	121·6 (2)		121·3 (3)
C(9a)–N(10)–C(16)	118·5 (2)		119·7 (3)
C(16)–N(10)–C(10a)	119·9 (2)		119·0 (3)
C(4a)–C(10a)–N(10)	117·1 (2)		118·5 (3)
C(4a)–C(10a)–N(1)	123·4 (3)		120·9 (3)
N(10)–C(10a)–N(1)	119·3 (3)		120·4 (2)
		N(5)–C(19)–C(18)	103·9 (3)
		C(19)–C(18)–O(17)	105·0 (3)
		C(4a)–O(17)–C(18)	108·4 (2)

both compounds were those given by Doyle & Turner (1968) for neutral C, N and O, and by Stewart, Davidson & Simpson (1965) for H.

(*c*) A direct-methods solution was also tried for (II) and (III); several attempts carried out with *MULTAN* did not show any feature which could help the structure interpretation. A different approach will be tried in the future.

Description of the structure

(*a*) 4a-*Isopropyl*-3-*methyl*-4a,5-*dihydrolumiflavin* (I)

The molecule is approximately planar, the angle between the two moieties divided by the N(5)–N(10) axis being only 2·3°. The least-squares plane calculated using all the atoms common to the isoalloxazine skeleton and excluding C(4a) shows that this atom is considerably displaced, its distance from the plane being 0·39 (3) Å. All other atoms lie within 0·1 Å of the mentioned plane (Table 6). The value of 1·304 (4) Å for the double bond between N(1) and C(10a) (Table 4) is identical with that reported by Norrestam (1972) for 4a-allyl-3,5,7,8,10-pentamethyl-4a,5-dihydroisoalloxazine and in good agreement with the values published for oxidized flavins (Norrestam & Stensland, 1972; Wang & Fritchie, 1973). The presence of an sp^3-hybridized atom, C(4a), is reflected in the C(10a)–C(4a) and C(4a)–N(5) bond lengths [1·509 (4) and 1·447 (3) Å respectively], which are longer than the values reported for 1,5-dihydro derivatives. The C(4a)–C(17) distance [1·580 (4) Å] in the isopropyl residue is significantly longer than that typical of a single C–C bond, and should be related to the pronounced lability of alkyl residues bound to

Table 6. *Atomic displacements* (Å) *from some significant least-squares planes of* (I) *and* (IV)

The general equation is $lx + my + nz + d = 0$, $D = (l^2 + m^2 + n^2)^{1/2}$, $\cos\alpha = -l/D$, $\cos\beta = -m/D$, $\cos\gamma = -n/D$.

Plane *A*: N(1), C(2), N(3), C(4), C(10a)
Plane *B*: N(1), C(2), N(3), C(4), C(4a), N(5), N(10), C(10a)
Plane *C*: N(5), C(5), C(6), C(7), C(8), C(9), C(9a), N(10)

		l	*m*	*n*	*d*	cos α	cos β	cos γ
Plane *A*	(I)	−2·8091	−10·2569	12·7899	−2·8769	−0·2389	−0·7295	0·6464
	(IV)	10·0638	−0·8773	4·0819	−1·3637	0·7946	−0·0577	0·5331
Plane *B*	(I)	−3·4233	−10·2966	12·3139	−2·5230	−0·2912	−0·7323	0·6224
	(IV)	10·8252	−1·1269	3·3319	−1·4121	0·8547	−0·0741	0·4270
Plane *C*	(I)	−3·3805	−10·6678	11·6981	−2·3334	−0·2875	−0·7587	0·5913
	(IV)	10·7303	−2·5782	3·2578	−0·7479	0·8472	−0·1694	0·4174

Table 6 (*cont.*)

Deviations from planes (Å)

	Plane *A*		Plane *B*		Plane *C*	
	(I)	(IV)	(I)	(IV)	(I)	(IV)
N(1)	0·04	−0·08	0·03	0·00	0·04	−0·02
C(2)	−0·06	0·06	0·01	0·13	0·01	−0·02
N(3)	0·06	−0·02	0·14	−0·09	0·09	−0·32
C(4)	−0·03	−0·01	−0·01	−0·21	−0·11	−0·40
C(4a)	−0·39	0·52	−0·45	0·34	−0·54	0·29
N(5)			0·26	−0·01	0·13	−0·01
C(5a)			−0·13	−0·12	−0·27	0·02
C(6)			0·23	−0·21	0·06	−0·01
C(7)			0·24	−0·32	0·07	0·01
C(8)			0·14	−0·41	0·02	0·00
C(9)			0·04	−0·35	−0·03	−0·01
C(9a)			0·07	−0·21	−0·01	0·01
N(10)			0·08	−0·16	0·05	0·00
C(10a)	0·00	0·04	−0·07	0·00	−0·10	0·03
O(11)	−0·19	0·17	−0·06	0·34	−0·02	0·14
C(12)	0·26	−0·11	0·42	−0·19	0·37	−0·56
O(13)	0·10	−0·26	0·14	−0·56	−0·01	−0·83
C(14)			0·33	−0·31	0·11	0·08
C(15)			0·13	−0·55	0·02	0·04
C(16)			0·34	−0·32	0·36	−0·08
C(17)			−2·02	1·80	−2·12	1·73
C(18)			−2·46	2·28	−2·62	2·21
C(19)			−2·78	1·03	−2·84	0·97

Angles between planes *B* and *C*: (I) 2·3°, (IV) 5·5°.

C(4a) in 4a,5-dihydroflavins (Norrestam, 1972, and references therein; Ghisla *et al.*, 1973). The only atom potentially capable of hydrogen bonding is H(105). In fact, a short contact [3·03 (1) Å] is found between N(5) and O(13), in such a way that pairs of molecules are connected through two parallel hydrogen bonds (Fig. 3).

In various flavins and related compounds, keto groups involved in hydrogen bonding with water as solvent (Fritchie & Johnson, 1975; Brufani, Casini, Fedeli, Giacomello & Vaciago, 1966) and also with N—H groups (Wang & Fritchie, 1973) were frequently found. In the present case an H(105)—O(13) distance of 2·19 (2) Å and a N(5)—H(105)—O(13) angle of 164·3° were found. Such N(5)—H(105)—O(13) base pairing and formation of N(5)—H(105) hydrogen bridges in general should be unique for 4a-mono-substituted 4a,5-hydroflavins. The remaining intermolecular distances are consistent with loosened van der Waals interactions, the shortest distances being those between atoms O(11), C(12), C(16), C(17) and C(19).

(*b*) 4a,5-*Epoxyethano*-3-*methyl*-4a,5-*dihydrolumiflavin* (IV)

This molecule is similarly almost planar, the angle between the two planes intersecting along the N(5)—N(10) axis being 5·5°. C(4a) is the atom which is farthest from the r.m.s. plane calculated on the basis of the isoalloxazine atoms: the displacement is 0·52 (3) Å, and thus greater than in (I).

The geometric strains present around this atom are reflected in the distorsion of the tetrahedral angles (Table 5). Two bond lengths around C(4a) are significantly different from the values found in (I) and in 4a-allyl-3,5,7,8,10-pentamethyl-4a,5-dihydroisoalloxazine (Norrestam, 1972), while the C(4a)—C(10a) distance is much closer to the average of the respective values in these two compounds.

Other geometrical features of the isoalloxazine backbone of (IV) are very similar to those of (I).

Attention is drawn to the fact that in this structure as well as in (I) the C(9a)—N(10) distance is considerably longer than that found for oxidized flavins [1·388 (3) Å]. The value observed by Norrestam (1972) of 1·424 (2) Å for the same bond length could suggest that in 4a,5-dihydroflavins this distance is systematically longer than in the oxidized state; this could reflect a possible reactivity of this centre, as postulated by Hemmerich (1976), for flavin activation of oxygen involving positions C(4a) and C(9a). With reference to the packing, a slight tendency towards stacking is contrasted by the C(19)—C(18)—O(17) bridge which extends almost perpendicularly to the plane of the molecule (Fig. 4). The shortest contacts are found in fact between H atoms of the epoxyethano chain and the

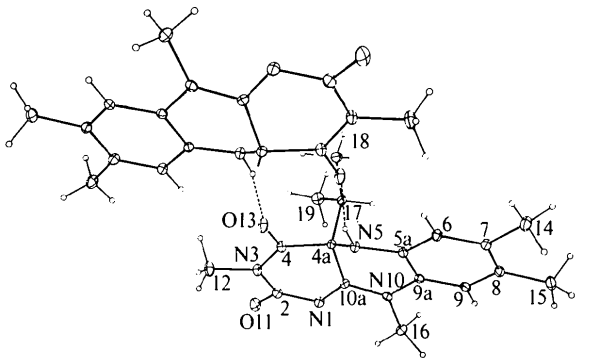


Fig. 3. *ORTEP* plot of two symmetry-related molecules of (I); ellipsoids are drawn at 10% probability. Hydrogen bonds are marked with dashed lines: in the upper molecule the isopropyl substituent has been omitted for clarity. Hydrogen atoms are represented as spheres corresponding to a temperature factor of 1·0 Å^2.

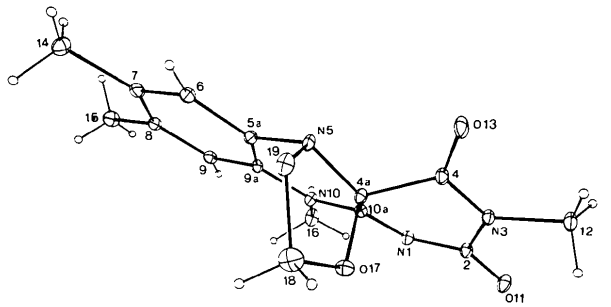


Fig. 4. *ORTEP* drawing of (IV); H(119) and H(219) have been omitted. All the atoms are drawn with the same conventions as in Fig. 3.

N(1) and N(5) atoms of the above molecule along **c**. Distances are, however, very close to the sum of the van der Waals radii according to Pauling (1948).

Discussion

The results detailed above indicate that the isoalloxazine moiety only slightly deviates from planarity in this 4a,5-dihydro form. In the enzyme *p*-hydroxybenzoate hydroxylase the flavin subunit of the coenzyme most probably is planar; similarly all (fully aromatic) oxidized flavin species investigated so far were found to be strictly planar (Kierkegaard *et al.*, 1971; Fritchie & Johnson, 1975). Similarly, this reduced (1,5-dihydro) enzyme (Entsch *et al.*, 1976) has absorption spectra typical of a 'planar type' 1,5-dihydroflavin species (Ghisla *et al.*, 1974; Dudley, Ehrenberg, Hemmerich & Müller, 1964).

If one assumes that the protein-bound (4a-OH) 4a,5-dihydrocoenzyme has a geometry similar to that of the models, as is also suggested by the similarity of the respective electronic spectra, then the coenzyme of *p*-hydroxybenzoate hydroxylase would not undergo major geometrical changes during redox catalysis. The 1,5-dihydroflavocoenzyme in *p*-hydroxybenzoate hydroxylase exhibits among reduced flavocoenzymes one of the highest reactivities toward molecular oxygen (Entsch *et al.*, 1976). A planar reduced coenzyme would be in keeping with the postulated relation between high oxygen reactivity and planarity of reduced flavin (see discussion in Hemmerich, 1976; Tauscher, Ghisla & Hemmerich, 1973).

In contrast to this, enzymes with lower oxygen reactivity (*cf.* old yellow enzyme, Ghisla *et al.*, 1974) exhibit spectra in the reduced state which are similar to those of non-planar 1,5-dihydroflavins. Attention is also drawn to the orientation of the C(4a) substituent in 4a,5-dihydroflavins. The C(4a) enzyme peroxide and the corresponding C(4a)—OH adduct in *p*-hydroxybenzoate hydroxylase, which have closely similar absorption spectra (Entsch *et al.*, 1976), would thus have a structure in which the substituent is perpendicular to the reduced flavin plane. This sets precise steric requirements for the location of substrate in the ternary complex preceding hydroxylation. The small spectral difference observed with 4a-OH and 4a-OOH model flavins (Ghisla *et al.*, 1977; Kemal & Bruice, 1976) also probably originates from different electronic interactions rather than from geometrical differences.

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The Crystal and Molecular Structure of *O*-Diazoacetyl-L-serine (Azaserine) at 21°C and 0°C: Model Error and Deterioration

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The crystal structure of *O*-diazoacetyl-L-serine has been determined from the intensities of 1377 unique reflections measured with an automated diffractometer at 21(±2)°C. The compound crystallizes in space group $P2_1$ with $a = 15{\cdot}291\ (2)$, $b = 5{\cdot}251\ (1)$, $c = 9{\cdot}523\ (2)$ Å, and $\beta = 96{\cdot}52\ (2)°$ with $Z = 4$, two molecules per asymmetric unit. The structure was refined by full-matrix least-squares techniques to a conventional R value of 0·064 for the 1135 reflections greater than $2\sigma(I)$. The average deterioration, based on six monitor reflections, was ~40% with a range of ~30% over the course of the data collection. Substantial inconsistencies between the two molecules (*A* and *B*) in the asymmetric unit indicated serious problems with the model (equivalent bond lengths differing by as much as 0·099 Å, 4·5 times the standard deviation of the difference) which were thought to be related to the deterioration problem. A second data set (1352 unique reflections) was collected with a new crystal at 0 (±1)°C: $a = 15{\cdot}234\ (2)$, $b = 5{\cdot}2652\ (8)$, $c = 9{\cdot}527\ (2)$ Å, and $\beta = 96{\cdot}33\ (1)°$, deterioration ~10% with a range of ~8%. The model refined to an R value of 0·039 for the 1216 reflections greater than $2\sigma(I)$ and precision and accuracy as judged by comparing bond lengths in molecules *A* and *B* were both substantially improved. The major difference between molecule *A*, which resembles glutamine, serine, and *O*-serine phosphate, and molecule *B* is a conformational one and is primarily due to the 76·1° difference in the χ^2[C(2)–C(3)–O(3)–C(4)] dihedral angle. The C–N and N–N distances in the diazoacetyl group are 1·312 and 1·128 Å, respectively, and the C–N–N angle is 178·7°.

Introduction

O-Diazoacetyl-L-serine (azaserine) is a glutamine antagonist which inhibits purine biosynthesis (Levenberg, Melnick & Buchanan, 1957). Buchanan and coworkers (French, Dawid, Day & Buchanan, 1963; Dawid, French & Buchanan, 1963; French, Dawid & Buchanan, 1963) have studied the reaction of azaserine with formyl-glycineamidoribonucleotide (FGAR) amidotransferase, one of the enzymes in the biosynthetic pathway, in detail. Apparently, azaserine resembles glutamine structurally and, therefore, fits into the active site of the enzyme easily. Once in the active site, azaserine alkylates an 'active' sulfhydryl group in the enzyme. A related compound, 6-diazo-5-oxo-L-norleucine (DON), resembles glutamine and inhibits FGAR amidotransferase in a manner similar to azaserine (Levenberg, Melnick & Buchanan, 1957). DON also inhibits glutamine phosphoribosyl pyrophosphate amidotransferase, another enzyme in the pathway, although azaserine is relatively inactive toward this enzyme (Hill & Bennett, 1969). Glutamine