

Electronic Structure and Spectra of Lumiflavin Calculated by a Restricted Hartree-Fock Method

BARBRO GRABE

Institute of Theoretical Physics, University of Stockholm, Stockholm, Sweden

Calculations are made on the lumiflavin molecule in the oxidized and reduced states and on the half-reduced radical in three different dissociation states. A semi-empirical restricted Hartree-Fock SCF-MO-LCAO-CI method in the Pariser-Parr-Pople approximation is used. The parameters are those determined in a series of papers¹⁻⁵ from this institute. The results include bond orders, bond lengths, charges on the atoms, ionization potentials, and spectra.

I. INTRODUCTION

In the large group of flavoproteins the riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) is an integral part of the prosthetic group. Substituted isoalloxazines have therefore been the subject of calculations by several persons. The results given in the present paper have been obtained by a semi-empirical method in the Pariser-Parr-Pople approximation (PPP) on lumiflavin (7,8,10-trimethylisoalloxazine), where thus the ribityl in riboflavin is replaced by methyl. The method is a SCF-MO-LCAO-CI method implying zero-differential overlap (ZDO), where only π -electrons are taken into consideration explicitly. The parameters used for carbon, methyl, nitrogen, and oxygen are those given in a series of papers.¹⁻⁵ This paper is an application of these parameters.

The investigation includes calculations on the molecule in the oxidized and reduced states and on the half-reduced radical in three different dissociation states. Furthermore, a calculation on the reduced molecule and on the radical have been made where a hydrogen is bound to O(2') instead of to N(1). (For the numbering see Fig. 1.)

The investigation includes electron densities, bond orders, bond lengths, ionization potentials, singlet transitions, oscillator strengths, and the polarization direction of the transition moments. Since the method used is a restricted Hartree-Fock method the spin densities obtained are not given here. The intention is to make further calculations using an unrestricted Hartree-Fock method, *i.e.* to use different orbitals for electrons with α and β spins. Spin densities will be reported and discussed in that context in a coming paper.

II. METHOD

The details of the method are given in the above-mentioned series of papers,¹⁻⁵ only some notations and equations are given here.

$$S_{\mu\nu} = (\mu|S|\nu) = \delta_{\mu\nu} \quad (1)$$

$$\alpha_{\mu} = (\mu|H^{\text{core}}|\mu) = W_{\mu} - (n_{\mu} - 1)\gamma_{\mu\mu} - \sum_{\mu \neq \nu} n_{\nu}\gamma_{\mu\nu} \quad (2)$$

$$\beta_{\mu\nu} = (\mu|H^{\text{core}}|\nu) \begin{cases} \neq 0, & \text{when } \mu \text{ and } \nu \text{ are neighbours} \\ = 0, & \text{when } \mu \text{ and } \nu \text{ are non-neighbours} \end{cases} \quad (3)$$

$$(\alpha\beta|1/r_{12}|\mu\nu) = \delta_{\alpha\mu}\delta_{\beta\nu}\gamma_{\mu\nu} \quad (4)$$

n_{ν} is the number of electrons that should be added to the core to give the neutral atom. Furthermore,

$$W_{\mu}(\text{P}, \text{Q} \dots) = W_{\mu}^{\circ} + \sum_{\substack{\text{P over} \\ \text{all bonded} \\ \text{atoms}}} [\Delta W_{\mu}^{\circ}(\text{P}) + \delta_{\mu\text{P}}^{\text{w}}(R_{\mu\text{P}} - R_{\mu\text{P}}^{\circ})] \quad (5)$$

$$\beta_{\mu\nu} = \beta_{\mu\nu}^{\circ} + \delta_{\mu\nu}^{\beta}(R_{\mu\nu} - R_{\mu\nu}^{\circ}), \quad \text{between nearest neighbours} \quad (6)$$

$$\gamma_{\mu\nu} = \gamma_{\mu\nu}^{\circ} + \delta_{\mu\nu}^{\gamma}(R_{\mu\nu} - R_{\mu\nu}^{\circ}), \quad \text{between nearest neighbours} \quad (7)$$

Semi-empirical values are used for most of the quantities above, see Table 1. The values are the same as those given in the series "Semi-empirical parameters in π -electron systems",¹⁻⁵ where the parameter choice is discussed. $R_{\mu\text{P}}$ and $R_{\mu\nu}$ are distances between the atoms, R° 's are suitably chosen standard values (see Table 1). The integrals $\gamma_{\mu\nu}$ for non-neighbours have been calculated by the ball approximation. $\gamma_{\mu\mu}$ is the value of the one-center two-electron integral.

An assumed structure must be used as input. From the bond orders between nearest neighbours obtained in the calculation, new bond lengths have been calculated from the relations

$$R_{\mu\nu}(\text{CC}) = 1.517 - 0.18 p_{\mu\nu} \quad (8)$$

$$R_{\mu\nu}(\text{CN}) = 1.458 - 0.18 p_{\mu\nu} \quad (9)$$

$$R_{\mu\nu}(\text{CO}) = 1.365 - 0.18 p_{\mu\nu} \quad (10)$$

The calculations were repeated with the obtained values as input and the procedure was continued until self-consistency was achieved. The final values of $R_{\mu\nu}$ never deviated from the previous cycle more than ± 0.003 Å. The distance between the CH_3 group and its nearest neighbour has not been varied.

The bond lengths obtained for the oxidized and the reduced forms have been compared with values experimentally determined.⁶⁻⁹

According to experimental results⁸ the reduced molecule is bent along the line through N(5) and N(10), the two planes intersecting at an angle about 150° . In the present calculation the molecule is assumed to be planar. This approximation only changes the β values with a factor 0.87 ($\cos 30^{\circ}$).

The SCF-MO's have been evaluated by means of a data machine programme written by B. Roos and M. Sundbom. This programme also calculates the energy levels of the excited states by mixing all or a selection of the configurations (max. 140 configurations) obtained from single excitations. Oscillator strengths for the dipole transitions according to formula by Mulliken and Rieke¹⁰ and eigenvectors for the multi-configurational problem are also obtained directly from the computer. The computer IBM 360/75 at Stockholm's datacentral has been used for the calculations.

The vertical ionization potential, IP, for an electron in a molecule with closed shells is equal to $-\varepsilon$, ε being the orbital energy obtained by the data machine programme. In the radicals the relation is somewhat more complicated. For the odd electron

$$\text{IP}_0 = -\varepsilon_0 + 1/2(\phi_0 | K_0 | \phi_0) \quad (11)$$

where ϕ_0 is the wave function and K_0 the exchange integral for the open shell. For the electrons in the closed shell l

$$\text{IP}_l^1 = -\varepsilon_l + 5/2(\phi_l | K_0 | \phi_l) \quad (\text{singlet}) \quad (12)$$

and

$$\text{IP}_l^3 = -\varepsilon_l + 1/2(\phi_l | K_0 | \phi_l) \quad (\text{triplet}) \quad (13)$$

For comparison of the stability of the keto form and the enol form (H bound to N(1) or to O(2')) it is the difference in total bond energy $E_{\text{bond}}^\pi + E_{\text{bond}}^\sigma$ between the two forms that is significant.

E_{bond}^π is determined for each form according to

$$E^\pi + E^{\text{core}} - \sum_{\mu} [n_{\mu} W_{\mu} - (n_{\mu} - 1) \gamma_{\mu\mu}] = -E_{\text{bond}}^\pi \quad (14)$$

where E^π is the π electron energy calculated by the data machine program, E^{core} is the repulsion between the core parts of the molecule, and the sum \sum , denoted E_{atom}^π , is the energy of the π electrons when they are localized giving neutral molecules.

$$E^{\text{core}} = \frac{1}{2} \sum_{\mu} \sum_{\nu \neq \mu} n_{\nu} \gamma_{\nu\mu} \quad (15)$$

For E_{bond}^σ only the difference in bond energy between the two forms has been estimated. The difference includes the difference in energy between a N-H bond and an O-H bond and the change in σ bond energy due to changes in bond lengths. For the determination of these two parts, values on bond energies and force constants have been taken from Cottrell.¹¹ The estimated values are very approximate.

III. NUMBERING OF THE ATOMS AND THE NOTATION OF THE MOLECULES AND RADICALS

For the numbering of the atoms see Fig. 1. The following notations of the molecules and radicals are used in order to be in accordance with, *e.g.*, the notations on flavin used by Ehrenberg and Hemmerich.¹²

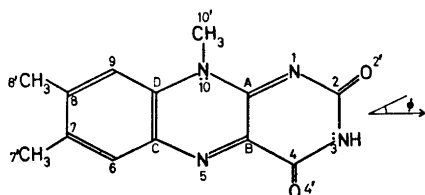
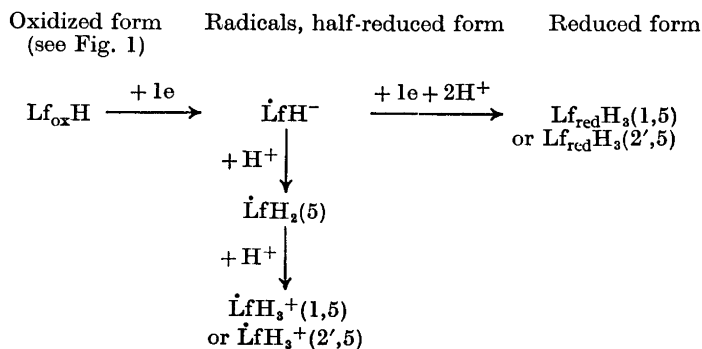


Fig. 1. Lumiflavin in the oxidized state to show the numbering of the atoms and the polarization angle ϕ .



The numbers in parenthesis indicate to which atoms the hydrogen atoms are bound. In all the cases a hydrogen atom is bound to N(3).

IV. RESULTS

Bond length. (1) The calculated bond lengths have been compared with experimental ones. For the oxidized state comparisons have been made with experimental values for 3,7,8,10-tetramethylisoalloxazine,⁶ thus a molecule methyl-substituted also at N(3), see Table 2. The calculated bond distances and the experimental ones differ more than 0.02 Å only for the C(2)–N(3) bond and the C–O bonds. Also values for 9-bromo-3,7,8,10-tetramethylisoalloxazine monohydrate⁷ are given in Table 2.

For the reduced state $\text{Lf}_{\text{red}}\text{H}_3(1,5)$ comparisons have been made with 9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisoalloxazine,⁸ see Table 2, thus more substituted than the calculated molecule. The only significant difference between experimental and calculated values is for C(2)–O(2') (experimental 1.19 and calculated 1.25 Å). Four other values differ by 0.02–0.03 Å, but this is of minor importance. In other reduced flavin compounds, *e.g.* 5-acetyl-3,7,8,10-tetramethyl-1,5-dihydroisoalloxazine,⁹ these distances are in better agreement with the calculated values. As mentioned above, the bent form of the reduced molecule is not taken into consideration in the calculations.

(2) Some observations can be done concerning the relations between the bond distances in the different states of the calculated molecule.

Going from the oxidized to the reduced state three bond lengths change more than the others, as could be expected, *i.e.* C(A)–N(1) and C(B)–N(5)

Table 2. Bond length in Å.

Bond	Exp.			Calculated					Exp.	
	Oxidized ^{a,b}	Lf _{ox} H	ĪFH ⁻	ĪFH ₂ (5)	ĪFH ₃ ⁺ (1,5)	ĪFH ₃ ⁺ (2',5)	Lf _{red} H ₃ (1,5)	Lf _{red} H ₃ (2',5)	Reduced ^{c,d}	
A1	1.303 ^a	1.322	1.343	1.324	1.384	1.369	1.398	1.372	1.391 ^c	1.377 ^d
12	1.368	1.381	1.358	1.380	1.388	1.325	1.377	1.319	1.367	1.363
22'	1.207	1.243	1.262	1.244	1.236	1.305	1.246	1.319	1.193	1.223
23	1.410	1.387	1.376	1.380	1.378	1.372	1.374	1.377	1.391	1.358
34	1.362	1.366	1.370	1.367	1.369	1.374	1.376	1.373	1.380	1.413
44'	1.219	1.212	1.258	1.249	1.237	1.241	1.243	1.251	1.223	1.226
4B	1.483	1.487	1.434	1.447	1.458	1.450	1.448	1.440	1.432	1.419
AB	1.446	1.438	1.414	1.438	1.391	1.400	1.377	1.388	1.350	1.362
B5	1.300	1.313	1.374	1.380	1.383	1.382	1.407	1.404	1.385	1.433
5C	1.372	1.382	1.379	1.405	1.389	1.391	1.405	1.405	1.394	1.425
DC	1.405	1.418	1.418	1.407	1.412	1.411	1.409	1.411	1.404	1.393
C6	1.406	1.403	1.407	1.404	1.414	1.413	1.399	1.398	1.370	1.384
67	1.371	1.369	1.400	1.396	1.388	1.388	1.402	1.403	1.386	1.394
77'	1.499	1.505							1.484	1.517
78	1.421	1.415	1.397	1.404	1.417	1.416	1.396	1.396	1.401	1.389
88'	1.502	1.516							1.511	1.507
89	1.376	1.411	1.403	1.396	1.389	1.389	1.403	1.403	1.402	1.395
9D	1.397	1.416	1.402	1.405	1.411	1.412	1.397	1.398	1.380	1.392
10D	1.380	1.418	1.391	1.400	1.394	1.391	1.413	1.406	1.438	1.424
10A	1.360	1.363	1.396	1.388	1.378	1.380	1.397	1.399	1.386	1.360
10, 10'	1.466	1.479							1.490	1.458

^a Norrestam-Stensland⁶ 3,7,8,10-tetramethylisalloxazine, *Acta Cryst.* **B 28** (1972) 440. ^b v. Glehn-Norrestam⁷ 9-bromo-3,7,8,10-tetramethylisalloxazine monohydrate, *Acta Chem. Scand.* **26** (1972) 1490. ^c Norrestam-v. Glehn⁸ 9-bromo-1,3,7,8,10-pentamethyl-1,5-dihydroisalloxazine, *Acta Cryst.* **B 28** (1972) 434. ^d Norrestam-Kierkegaard-Stensland-Torbjörnsson⁹ 5-acetyl-3,7,8,10-tetramethyl-1,5-dihydroisalloxazine, *Chem. Comm.* **1969** 1250.

increase and C(A)–C(B) decreases. The only other distances which have changed more than 0.02 Å are N(5)–C(C) (0.028 Å) and N(10)–C(D) (0.025 Å). The distances in the benzene ring, C(C)–C(D) excluded, have alternately increased and decreased, so that while in the oxidized state the bond lengths are alternating long and short (largest difference 1.387 to 1.416 Å), in the reduced molecule the bond lengths are approximately equal (variation 1.396 to 1.403 Å).

The bond lengths in the radicals do not generally vary regularly in relation to the bond lengths in the oxidized and the reduced states. In the series $\dot{\text{L}}\text{fH}^- - \dot{\text{L}}\text{fH}_2 - \dot{\text{L}}\text{fH}_3^+(1,5)$ all the bonds in the pyrimidine ring except C(2)–N(3) and N(3)–C(4) change significantly. The other bonds in the molecule change only a little except N(5)–C(C) and the C–O bonds. The changes in the benzene ring are, however, such that the bonds in $\dot{\text{L}}\text{fH}^-$ and $\dot{\text{L}}\text{fH}_2$ are approximately equal while the bonds in both $\dot{\text{L}}\text{fH}_3^+$ radicals are alternating long and short as in the oxidized molecule.

The only bond lengths which are changed more than 0.01 Å when H is bound to O(2') instead of to N(1) are C(A)–N(1), N(1)–C(2) and C(2)–O(2'), see Table 2.

Charges on the atoms due to the π -electrons. The charges on the atoms are given in Table 3. The most negatively charged atoms in the molecules are the oxygen atoms, when they are in the keto form. The most negatively charged nitrogen is N(1), except in $\dot{\text{L}}\text{fH}_3^+(1,5)$ and $\text{L}\text{f}_{\text{red}}\text{H}_3(1,5)$, where all the nitrogens are positively charged.

The differences in charge between the oxidized and the reduced states for the atoms where the changes are largest are given in Table 4. From Table 3 it is also seen that the differences in charge between $\text{L}\text{f}_{\text{red}}\text{H}_3(1,5)$ and $\text{L}\text{f}_{\text{red}}\text{H}_3$

Table 3. Net charges on the atoms due to the π -electrons in units of electronic charge e .

Atom	$\text{L}\text{f}_{\text{ox}}\text{H}$	$\dot{\text{L}}\text{fH}^-$	$\dot{\text{L}}\text{fH}_2(5)$	$\dot{\text{L}}\text{fH}_3^+(1,5)$	$\dot{\text{L}}\text{fH}_3^+(2',5)$	$\text{L}\text{f}_{\text{red}}\text{H}_3(1,5)$	$\text{L}\text{f}_{\text{red}}\text{H}_3(2',5)$
A	0.084	0.038	0.031	-0.011	0.014	-0.073	-0.029
N1	-0.247	-0.305	-0.218	0.284	-0.251	0.264	-0.216
2	0.341	0.289	0.339	0.350	0.125	0.338	0.017
O2'	-0.643	-0.741	-0.650	-0.620	0.100	-0.675	0.065
N3	0.361	0.357	0.369	0.367	0.384	0.341	0.356
4	0.378	0.319	0.314	0.326	0.341	0.343	0.330
O4'	-0.647	-0.729	-0.664	-0.606	-0.634	-0.651	-0.694
B	0.053	-0.154	-0.012	-0.068	-0.021	-0.112	-0.049
N5	-0.031	-0.128	0.328	0.472	0.434	0.187	0.195
C	-0.037	0.027	-0.029	-0.004	-0.010	0.004	-0.001
6	-0.005	-0.069	-0.089	-0.081	-0.084	-0.098	-0.105
7	0.024	-0.039	0.038	0.122	0.110	0.022	0.009
CH ₃ 7'	0.020	0.015	0.021	0.030	0.029	0.019	0.018
8	0.077	-0.025	0.044	0.132	0.116	0.010	0.005
CH ₃ 8'	0.023	0.016	0.021	0.031	0.029	0.018	0.018
9	-0.124	-0.166	-0.095	-0.090	-0.094	-0.109	-0.117
D	0.034	0.037	-0.026	-0.015	-0.013	-0.031	-0.016
N10	0.332	0.254	0.273	0.368	0.414	0.198	0.209
CH ₃ 10'	0.007	0.003	0.005	0.012	0.014	0.003	0.003

Table 4. The difference between net charge in the reduced state and the charge in the oxidized state on the atoms where the changes are largest.

	C(A)	N(1)	C(2)	O(2')	C(B)	N(5)	C(6)	N(10)
$\text{Lf}_{\text{red}}\text{H}_3(1,5) - \text{Lf}_{\text{ox}}\text{H}$	-0.16	0.51			-0.17	0.22	-0.09	-0.13
$\text{Lf}_{\text{red}}\text{H}_3(2',5) - \text{Lf}_{\text{ox}}\text{H}$	-0.11		-0.32	0.71	-0.10	0.23	-0.10	-0.12

(2',5) are large only at N(1), C(2) and O(2') and to some extent at C(A), O(4'), and C(B).

At most of the atoms the charges in the radicals are between the charge values in the oxidized and the reduced states or at least approximately near one of them.

At some atoms this is not the case, the charges varying more irregularly when passing the different oxidation states. This is of course to a great extent due to the fact that some of the radicals are charged. See some examples in Fig. 2. Especially significant are

- (1) the large positive charge on N(5), N(10) and to some extent on C(7) and C(8) in $\dot{\text{L}}\text{fH}_3^+(1,5)$ and $\dot{\text{L}}\text{fH}_3^+(2',5)$ and on N(5) in $\dot{\text{L}}\text{fH}_2$.
- (2) the large negative charge on N(5) and C(B) and to some extent on O(2'), O(4') and C(9) in $\dot{\text{L}}\text{fH}^-$ and the negative charge on C(7) and C(8) in this radical.

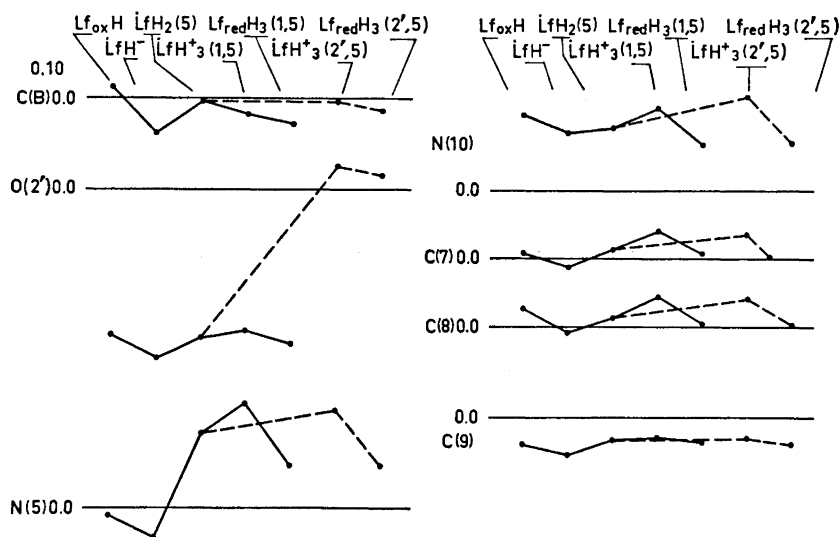


Fig. 2. Charges on some of the atoms in the different oxidation and dissociation states.

Table 5. Net charges added for larger parts of the molecule due to π -electrons in units of electronic charge e .

	$Lf_{ox}H$	$\dot{L}fH^-$	$\dot{L}fH_2(5)$	$\dot{L}fH_3^+(1,5)$	$\dot{L}fH_3^+(2',5)$	$Lf_{red}H_3(1,5)$	$Lf_{red}H_3(2',5)$
Pyrimidine ring with A and B	-0.320	-0.926	-0.491	0.022	0.058	-0.225	-0.220
Middle part: N5, N10 and CH_310'	0.308	0.129	0.606	0.852	0.862	0.388	0.407
Benzene ring with C and D	0.012	-0.204	-0.115	0.125	0.083	-0.165	-0.189
Pyrimidine part without A and B (1,2,2',3,4,4')	-0.457	-0.810	-0.510	0.101	0.065	-0.040	-0.142
Middle ring with A, B, C and D	0.442	0.077	0.570	0.754	0.832	0.176	0.312
Benzene ring without C and D (6,7,7',8,8',9)	0.015	-0.268	-0.060	0.144	0.106	-0.138	-0.172
A+B	0.137	-0.116	0.019	-0.079	-0.007	-0.185	-0.078
C+D	-0.003	0.064	-0.055	-0.019	-0.023	-0.027	-0.017

It may be of interest to see how the charges are dislocated in the different states and therefore the charge on larger parts of the molecule are given in Table 5. From this table it is seen that the pyrimidine ring compared to the other parts of the molecule is more negatively charged in the neutral molecules and in $\dot{L}fH^-$ and less positively charged in the positively charged radicals. The same is valid also when the pyrimidine ring is considered without C(A) and C(B) except in the reduced state. The middle part of the molecule is always positively charged. The relatively large variation in charge of the benzene ring is remarkable, the charge on C(C) + C(D), however, being relatively constant. In the reduced state the π -electrons are dislocated more towards C(A) + C(B) and the benzene ring than in the oxidized state.

Table 6. For notations, see paragraphs II and III.

	$Lf_{ox}H$	$\dot{L}fH^-$	$\dot{L}fH_2(5)$	$\dot{L}fH_3^+(1,5)$	$\dot{L}fH_3^+(2',5)$	$Lf_{red}H_3(1,5)$	$Lf_{red}H_3(2',5)$
Dipole moment, Debye							
μ^π	8.92	8.85	8.84	11.45	4.42	5.30	5.13
$\mu^{\pi x}$	-8.64	-6.04	-8.82	-11.44	-4.36	-4.01	2.01
$\mu^{\pi y}$	2.22	6.48	0.60	-0.61	0.72	3.47	4.72
Energies in ground state							
E^π , a.u.	-50.264	-50.258	-54.181	-58.132	-57.244	-58.208	-57.289
E^{core} , a.u.	30.521	30.475	31.768	33.059	32.741	32.944	32.629
E_{atom}^π , a.u.	-11.779	-11.787	-12.459	-13.159	-13.152	-13.140	-13.137
E_{bond}^π , a.u.	7.96	8.00	9.95	11.91	11.35	12.12	11.52
» kcal/mol $\times 10^{-2}$	50	50	62	75	71	76	72
				$\dot{L}fH_3^+(1,5) - \dot{L}fH_3^+(2',5)$		$Lf_{red}H_3(1,5) - Lf_{red}H_3(2',5)$	
ΔE_{bond}^π				0.56 a.u. = 353 kcal/mol		0.60 a.u. = 376 kcal/mol	
ΔE_{bond}^σ				ca. -27 kcal/mol		ca. -25 kcal/mol	
ΔE_{bond}^{total}				ca. 330 kcal/mol		ca. 350 kcal/mol	

Dipole moments and bond energies. The dipole moments and the π bond energies are given in Table 6. The estimated differences in σ bond energy between the keto forms and the enol forms are also given. It is seen that for the reduced $\text{Lf}_{\text{red}}\text{H}_3$ as well as for the cation radical $\dot{\text{L}}\text{fH}_3^+$ the bond energy is larger for the keto form than for the enol form, the differences being estimated to about 350 resp. 330 kcal/mol. That the keto form is the more stable one is in agreement with experimental findings.

Ionization potentials. In Table 7 the calculated vertical ionization potentials are given. For the radicals the first IP implies the ionization of the

Table 7. Calculated vertical ionization potentials in eV. Notation o is ionization of the odd electron, t=triplet, s=singlet, the state after ionization.

$\text{Lf}_{\text{ox}}\text{H}$	$\dot{\text{L}}\text{fH}^-$	$\dot{\text{L}}\text{fH}_2(5)$	$\dot{\text{L}}\text{fH}_3^+(1,5)$	$\dot{\text{L}}\text{fH}_3^+(2',5)$	$\text{Lf}_{\text{red}}\text{H}_3(1,5)$	$\text{Lf}_{\text{red}}\text{H}_3(2',5)$
	o 2.75	o 7.84	o 12.75	o 11.87	6.53	5.88
7.99	t 3.32 s 4.88	t 7.72 s 9.51	t 12.83 s 14.68	t 12.02 s 14.40	8.61	7.96
9.09	t 4.15 s 5.85	t 9.04 s 10.67	t 13.17 s 15.06	t 12.47 s 14.30	8.91	8.34
9.39	t 4.76 s 5.03	t 9.20 s 10.04	t 13.50 s 13.65	t 13.27 s 14.16	9.78	9.15
10.00	t 5.64 s 6.43	t 9.87 s 10.40	t 14.21 s 15.22	t 13.95 s 14.48	10.15	10.01

unpaired electron. Ionization of an electron from a closed shell in the radicals gives a triplet or a singlet, the two cases are denoted t and s in the table.

As far as the author knows, no experimental values on IP's for isoalloxazine or lumiflavin have been published. The calculated value of the first IP in the oxidized state of lumiflavin (7.99 eV) seems, however, to be reasonable in comparison to other molecules consisting of three fused rings as anthracene (IP 7.23–7.55 eV¹³) and acridine (IP 7.78 eV¹³). The consecutive size order of the ionization potentials of the various neutral and charged molecules and radicals seems to be very reasonable.

Electronic spectra. In Table 8 a–e calculated values on transition energies, oscillator strengths and direction of the transition moments are given, viz singlet-singlet $\pi-\pi^*$ transitions for the molecules with paired spins in the ground state and doublet-doublet $\pi-\pi^*$ transitions for the radicals with one unpaired electron in the ground state. Experimental values obtained by different workers are also given, in some cases estimated from reported experimental curves.

If the calculated spectra are compared with the experimental ones two facts are to be noticed.

Table 3a. Electronic singlet-singlet transitions below 60 kK of oxidized lumiflavin, Lf_{ox}H.

Calculated		Observed absorption															
ν, kK	f	Riboflavin in dioxane Koziol ¹⁴		Riboflavin in H ₂ O, phosphate buffer, pH 7.0 Whitby ¹⁵		Riboflavin in H ₂ O Penzer and Radda ¹⁶		Flavin in H ₂ O, phosphate buffer pH 7.0 Dudley <i>et al.</i> ¹⁷		Glucose oxidase Palmer and Massey ¹⁸							
		pol. λ, nm	max. ν, kK	min. ν, kK	max. λ, nm	min. ν, kK	max. λ, nm	min. ν, kK	max. λ, nm	min. ν, kK	max. λ, nm						
		$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$						
27.2	0.54	165	440	22.7	12.1	445	22.5	20 ^c	447	22.4	20 ^c	446	22.4	452	22.1	19 ^c	14.1
34.4	0.04	41	344	29.1	8.4	373	26.8	25.0	375	26.7	25	370	27.0	383	26.1	24.4	9.3
35.9	0.05	177						10.6									13.7
40.3	1.13	27	271	36.9	33.1	266	37.6	33	265	37.7	32	270	37.0	278	36.0	30 ^d	5.3
41.1	0.00							42 ^d									
44.9	0.17	36															
47.2	0.17	146															
49.4	0.50	163	224	44.6	31.0				220	45.5		230	43.5				
51.0	0.44	147															
51.6	0.09	20															
52.2	0.04																
55.1	0.06																
56.5	0.62	54															
57.5	0.15	59															
58.1	0.38	94															

^a The angle ϕ , see Fig. 1. ^b Estimated from curve in Palmer and Massey¹⁸ (in Fig. 2) and based on value 14.1 at 450 nm in Massey and Palmer.¹⁸
^c The start of the band. ^d End of the recorded curve.

Table 8c. Electronic doublet-doublet transitions below 50 kK of the lumiflavin radical $\dot{\text{L}}\text{fH}_2(5)$.

Calculated			Observed absorption			
			Neutral semiquinone of glucose oxidase, pH 6.3 Palmer and Massey ¹⁸			
ν , kK	f	pol. ϕ^a	max.		min.	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1} b$
			λ , nm	ν , kK	ν , kK	
11.4	0.08	176			15 ^c	0.3
19.8	0.01		~ 600	~ 17		1.9
22.8	0.00				18.5	1.7
24.5	0.04	31	450	22.2		5.5
26.2	0.00				24	5.1
26.8	0.07	21	382 (shoulder)	26.2		9.3
28.2	0.00					
31.5	0.11	165	360	27.8		10.1
35.2	0.11	166				
					30 ^d	7.5
37.7	0.02					
38.7	0.31	42				
40.1	0.05					
40.3	0.29	9				
41.6	0.08	174				
43.0	0.10	2				
43.7	0.00					
45.3	0.24	179				
46.1	0.12	151				
47.3	0.08	113				
47.9	0.05					
49.3	0.34	157				
49.8	0.05					

^a The angle ϕ , see Fig. 1 ^b Estimated from curve in Palmer and Massey¹⁸ and based on value 14.1 at 450 nm in Massey and Palmer.¹⁹ ^c The start of the band. ^d End of the recorded curve.

(1) To get as good agreement as possible it is necessary to displace the whole calculated spectrum, given in frequency units, between 3 and 5 kK towards smaller values as is seen in Tables 8 a–e. The calculated values should be compared with experimental data for the molecule in vapour phase. Experimental data available are, however, determined in solution. It is essential to make comparison with anthracene, a hydrocarbon with three fused rings, treated by Fischer-Hjalmars and Sundbom³ with the same method and parameters as in the present paper. For this molecule there are experimental data for the vapour phase as well as for solution. From Table 19 in Fischer-Hjalmars *et al.*³ it is seen that the calculated values on transition energies are about 2–4 kK higher than the experimental values for anthracene in vapour phase and 4–5 kK higher than those in heptane. Then the discrepancy be-

Table 8d. Electronic doublet-doublet transitions below 50 kK of two lumiflavin radicals $\dot{\text{L}}\text{fH}_3^+$.

Calculated						Observed	
$\dot{\text{L}}\text{fH}_3^+(1,5)$			$\dot{\text{L}}\text{fH}_3^+(2',5)$			Flavin semiquinone $\dot{\text{F}}\text{H}_3^+$ Ehrenberg and Hemmerich ¹²	
ν, kK	f	pol ϕ^a	ν, kK	f	pol ϕ^a	λ, nm	ν, kK
16.8	0.02		16.4	0.07	155		
18.9	0.03		19.3	0.07	36		
19.4	0.13	174	20.5	0.06	166	ca. 490	ca. 20 (max)
24.0	0.00		24.4	0.00			
26.5	0.00		26.4	0.01			
27.6	0.08	163	28.4	0.00		ca. 360	ca. 28 (max)
29.5	0.07	42	30.3	0.16	4		
31.2	0.01		32.9	0.05			
34.6	0.02		35.1	0.01			
38.5	0.01		36.8	0.08	14		
39.1	0.02		37.6	0.62	14		
40.6	0.01		40.2	0.00			
41.1	0.22	49	41.2	0.01			
42.1	0.44	14	43.0	0.01			
43.2	0.08	129	43.7	0.29	167		
43.8	0.26	178	45.3	0.05			
44.6	0.02		46.4	0.06			
45.8	0.22	146	47.2	0.02			
47.5	0.04		47.9	0.13	144		
47.8	0.09	178	48.6	0.14	159		
48.7	0.02		48.9	0.05			
49.4	0.02		49.7	0.06			

^a The angle ϕ , see Fig. 1.

tween calculated and experimental values for riboflavin seems to be due partly to the fact that the experimental values refer to solution, and partly to a property of our carbon parameters when used on molecules with three fused rings.

(2) In the calculated spectrum for the oxidized lumiflavin there is no real counterpart to the experimental band in the near UV (about 350 nm), only two weak transitions. It is to be observed that this band contrary to the other bands is significantly solvent sensitive. In fact, Koziol¹⁴ has pointed out that it is highly probable, that this absorption band represents an intramolecular complex. The present results support the assumption that this band does not originate from a simple electronic transition in the isolated molecule of the type calculated here. The experimental results for the radical anion $\dot{\text{L}}\text{fH}^-$ indicate that a similar peak due to a complex may occur also in this case.

The results of mixing configurations for the excited states show that very few transitions are pure. Thus except for $\text{Lf}_{\text{red}}\text{H}_3(2',5)$ there are at most one wave function for each molecule or radical, where there is a coefficient larger than 0.80. For $\text{Lf}_{\text{red}}\text{H}_3(2',5)$ the four lowest transitions each have one coefficient

Table 8e. Electronic singlet-singlet transitions below 60 kK of reduced lumiflavin Lf_{red}H₃.

Calculated						Observed absorption			
Lf _{red} H ₃ (1,5)		Lf _{red} H ₃ (2',5)					Flavohydroquinone Fl _{red} H ₃ in H ₂ O		
							Ehrenberg and Hemmerich ¹² (see also Hemmerich <i>et al.</i> ²¹)		
ν , kK	f	pol. ϕ^a	ν , kK	f	pol. ϕ^a	λ , nm	ν , kK	$\epsilon \times 10^{-3} \text{M}^{-1} \text{cm}^{-1}$	
21.5	0.06	112	21.7	0.05	67				
31.6	0.19	4	30.1	0.31	179	ca. 400	ca. 25 shoulder	ca. 2	
35.9	0.21	127	34.3	0.02		ca. 300	ca. 33 shoulder	ca. 6	
40.1	0.14	39	36.3	0.32	65				
43.2	0.61	11	40.3	0.80	174	250	40.0 max	ca. 30	
44.0	0.33	156	44.4	0.30	161				
47.1	0.02		47.5	0.16	141				
48.1	0.26	88	48.0	0.06					
48.6	0.05		49.8	0.00					
50.9	0.07	63	50.8	0.05					
53.8	0.80	105	53.8	0.65	84	204	49.0 max	ca. 30	
54.5	0.64	27	55.4	0.25	14				
55.0	0.21	68	55.8	0.18	174				
56.1	0.02		56.5	0.13	1				
56.8	0.16	24	56.8	0.08	71				
57.1	0.07	179	58.4	0.17	89				
59.8	0.30	97	58.9	0.18	62				
			59.6	0.08	60				

^a The angle ϕ , see Fig. 1.

In the experimental curve given by Ehrenberg and Hemmerich¹² the absorption ($\epsilon \times 10^{-3}$) increases continuously from about 1 at 22 kK (450 nm) to about 30 at 40 kK (250 nm). Further it is a minimum at 43.5 kK (230 nm), $\epsilon \times 10^{-3}$ about 12. According to Dudley *et al.*¹⁷ the intensity in the 500–700 nm range appears to be a measure of molecular planarity.

larger than 0.80. The details seem to be of minor interest so they are not published.

V. DISCUSSION

It is possible to choose different methods for the quantum chemical calculations. When the molecules are as large as in the actual case, however, it is not possible to use *ab initio* calculations. The CNDO (complete neglect of differential overlap) method, at least for such large molecules does not seem to give better results than the method used in spite of the fact that the data machine time is much longer. The method used is a restricted Hartree-Fock method in the Pariser-Parr-Pople (PPP) approximation. To get spin densities in the radicals which could then be compared with values calculated from ESR measurements, it is however necessary to use an unrestricted Hartree-Fock method. Such calculations have been started. In other respects the two methods, according to experience on smaller molecules, may give equivalent values.

Concerning the choice of semi-empirical parameters it can be said that the intention in our group has been to determine parameters that are useful not

only within a series of similar molecules but are more generally applicable. Although it cannot be expected that the consistency with experimental data will be as good as if the parameters were more specially determined, it seems in many cases to be of interest to have such generally determined parameters. It may be an improvement of our method if the parameter values on W and $\gamma_{\mu\mu}$ are made dependent on the charge of the atom. Such variations of the oxygen parameters in C–O bonds have been made in collaboration with Skancke²² on small molecules and similar variations of the nitrogen parameters are to be published. It is the intention to use these charge dependent parameters on the lumiflavin molecule.

When the oxidized form of lumiflavin is protonated to $Lf_{ox}H_2^+$, the second hydrogen is bound to N(1), but when LfH^- is protonated the second hydrogen goes to N(5). According to the present calculations the most negatively charged nitrogen is N(1) in both cases. It is, however, not only the size of the negative charge that determines the localization of the proton, but also the delocalization energy and the ground state energy of the formed molecule.

As mentioned above I intend to make further calculations, where W and $\gamma_{\mu\mu}$ are varied with the charge on the atom. This may change the electron distribution, so further discussion may wait for these new results.

Comparison with earlier calculations by other persons. Fox *et al.*²³ have made calculations on 7,8-dimethylisalloxazine in oxidized and reduced form with a semi-empirical SCF method in the PPP approximation but with another parameter set than the one used here. To get good agreement with experiments they have used different values for β if the atoms belong to a molecule with one, two, or three fused rings.²⁴ Their spectra for the oxidized molecule have the same general structure as those obtained here, *i.e.* they find only small absorption in the near UV (about 350 nm). This result supports the assumption, that the absorption in this region does not originate from a simple electron transition. Their calculated strong lines are nearer the experimental peaks than those obtained with our parameters. Their results being closer may result from their varying of β with the magnitude of the ring system.

They have made calculations on the reduced molecule under the assumption of a planar molecule as well as of a bent molecule. The experimental spectrum shows an increased absorption from about 22 kK to about 40 kK. In this respect the present calculation reproduces the spectrum considerably better than the calculations by Fox *et al.*²³ Unfortunately they have not published any transitions in the 50 kK region, where the experimental curve has a maximum which is reproduced in the present calculation.

Pill-Soon Song²⁵ has also made calculations on the oxidized state of 7,8-dimethylisalloxazine with a semi-empirical SCF method in the PPP approximation. He has especially studied the effect of the methyl groups and therefore treated these groups in four different manners. Unfortunately only the 1st and the 2nd electronic transitions are reported. For three of the calculations the 1st transition is at *ca.* 25 kK with the oscillator strength *ca.* 0.7 and the 2nd transition at *ca.* 31 kK with the oscillator strength *ca.* 0.13. Thus also here only a weak transition corresponds to the peak in near UV of the experimental spectrum. The angle between the transition directions was calculated to *ca.* 40°; the present calculation gives the value 55° (see Table 8a).

Fraga and Fraga²⁶ have made systematic calculations on large molecules, among others on the keto form of isoalloxazine. They have used a Hartree-Fock SCF method with configuration interaction. The 1st electronic transition is obtained at 28 kK, followed by three weak transitions and then a strong transition at 44 kK. This gives a less good agreement with experimental values than our method and the other methods discussed here.

There are several other calculations made with rather different methods, e.g. Hückel calculations, but they will not be discussed here. Calculations with unrestricted Hartree-Fock methods will be discussed when planned calculations with such a method have been carried out in this laboratory.

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