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A MINDO/3 MO Study of Oxidized Flavins

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The use of semi-empirical molecular orbital methods for the study of biologically important molecules has increased in recent years. The validity of well-known approximate Hartree–Fock molecular orbital methods such as MINDO/3 and MNDO still remains largely untested for biological molecules. Here we report its first application to the study of the electronic structures of isoalloxazines. Electronic charge distribution, variation of dipole moment, first ionization potential, electron affinity, orbital energies and electronic transitions are examined. Some of these results are compared with experiments. Good correlations with experiments were generally found in net atomic charge distributions, ionization potentials and electronic transitions. As a result of relatively good correlations with experiment application of the MINDO/3 method for extended study of biologically important coenzymes is promising.

Key words: Flavins – Isoalloxazines – **MINDO/3** – **NMR** – Energies – Dipole moments – Ionization potentials – Electron affinities – Semiempirical.

1. Introduction

Due to excessively prohibitive computational time, the application of the *ab initio* SCF (self-consistent field) H–F (Hartree–Fock) LCAO (linear combination of atomic orbitals) MO (molecular orbital) has been restricted to relatively small molecules. For this reason, semi-empirical molecular orbital methods have been

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favored over the *ab initio* method in order to study biological molecules. Among the most frequently used semi-empirical methods for biological systems are CNDO [1–3], SCF–PPP [4–11], EHMO [3, 12], and HMO [13, 14] methods, among which the CNDO method is the latest. However, we find that the use of the more recently developed, but well-accepted MINDO/3 method [15] for the study of the biological molecules has been seldom seen in the literature.

The advantage of both the MINDO/3 and MNDO [16] methods developed by Dewar et al. is manifold; with a single parameterization scheme, it yields simultaneous prediction of physical and chemical properties such as equilibrium geometry, dipole moment, ionization potential, normal vibration frequency and heat of formation, including other physical properties such as polarizability and hyperpolarizability [17–19] and nuclear magnetic and quadrupole coupling constant [20–22]. In addition, various reaction path studies with these methods have been found quite useful. More recently, the application of the methods have been extended even to organic polymer systems [23–25]. However, due to their inherent limitations: (1) Hartree–Fock method, (2) the neglect of 3- and 4-center integrals and (3) dependency of parameterization on experimental values, close scrutiny, particularly for largely untested biological molecules, is required. To the best of our knowledge there exist only a few MINDO applications [26, 27]. In the present study, we use MINDO/3 to study hither-to untested molecules in the isoalloxazine series, with particular attention to the electronic structure of these molecules.

Some of the previously reported semi-empirical calculations on flavin molecules have been biased by the particular choice of experimental parameters. The role of the σ electrons has only been explored to the extent of calculating the core potential and for the isoalloxazine molecule (excluding all methyl groups and the ribityl chain). This study, using MINDO/3, naturally includes the effect of the σ electrons with a bias. Thus all valence electrons are taken into account in the present study of lumiflavin, riboflavin, and the active sites of two flavodoxin flavoenzymes.

The molecular orbital study of flavin molecules was initially reported using the modified Hückel approximation for π electron systems [13]. Experimental parameters derived from studies on aromatic hydrocarbons, purines, and pyrimidines were used to approximate the bond distances and integrals. The calculations indicate that upon reduction of the flavin the orbitals redistribute themselves so that a high filled orbital is associated with the reduced isoalloxazine ring. In the reported charge density map of the isoalloxazine ring, N1 was shown to be more negative than N5, and N10, more positive than N3. By applying empirical resonance integrals values [14, 28] to the Hückel calculations, transition energies for FMN (flavin mononucleotide) were calculated and absorption maxima of FMN at 450 nm, of FMNH at 565 nm, and of $FMNH_2$ at 576 nm were predicted. Comparison of Hückel charge density maps for tryptophan and flavins led to the proposal that a reported tryptophan-FMN complex [29] was a charge transfer-complex [30]. Hückel MO calculations of the spin densities for different configur-

ations of phenothiazine, a three ring structure with similar properties to reduced isoalloxazine, predicted that phenothiazine and its free radical were folded along an axis through the central heteroatoms [31]. It was speculated that the reduced isoalloxazine ring was also folded. Recent crystallography studies [12] of reduced flavins have partially substantiated this prediction, although a study of semiquinone (free radical) riboflavin had indicated that it is planar [32].

Calculations of the π electrons of flavins by the Pariser–Parr–Pople (PPP) semi-empirical MO method represented an improvement in accuracy, though an increase in complexity [3, 5] [33–37]. Bond lengths for isoalloxazine tautomers were computed within 0.02 Å of the experimentally measured values. Calculated transition energies agreed closely with experimental values. The lowest triplet transition state for flavin was placed in the 700 to 750 nm range [38]. The calculated oscillator strength for the transition energy corresponding to an absorption maximum at around 350 nm was much lower than the experimental molar absorption coefficient. This result, along with solvent studies, has led to speculation that a $\sigma \rightarrow \pi^*$ transition might be involved in this band. Attempts to perform calculations on the semiquinone form of flavins were unsuccessful due to convergence failure.

All valence electron calculations of isoalloxazine using the CNDO and Extended Hückel Methods have been reported [2, 3]. Extended Hückel calculations, however, gave erroneous eigenvectors [3]. Using CNDO, the spin densities for the lowest triplet were shown to be concentrated on atoms N5, C4a, O4, N1, C9, and O2, in decreasing order. The CNDO calculations indicated that the lowest singlet-singlet transition of flavin is $\pi \rightarrow \pi^*$, while suggesting that the lowest triplet state could be $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ with n vibronic coupling. Second order perturbation applied to the results of a CNDO calculation resulted in several possible conformations for coenzyme FAD (flavin adenine dinucleotide) complexes [39]. Our emphasis in this paper is the applicability of the MINDO/3 method for the study of hither-to untested (by MINDO/3) biological molecules in the isoalloxazine series. We find good correlations with experiment, particularly in atomic charge distributions, ionization potentials and electronic transitions.

2. Computed Results and Discussions

In the use of the MINDO/3 Hartree–Fock method, geometry optimization is often recommended along with the usual SCF procedure. The optimization is to seek the minimum energy configurations in the field of hyperpotential interaction, simultaneously satisfying a SCF convergence criterion at the same configurations. The total molecular energy at the optimized geometry is the sum of the electronic energy E_e and nuclear repulsion energy E_n

$$E = E_e + E_n. \tag{1}$$

The electronic energy E_e is related to (obtained from) the converged Hartree–Fock elements $F_{\mu\nu}$. That is, for the choice of the spin-restricted Hartree–Fock

method [40], we have

$$E_e = \tfrac{1}{2} \sum_{\mu\nu} P_{\mu\nu}(H_{\mu\nu} + F_{\mu\nu}) \tag{2}$$

where $P_{\mu\nu}$ is the electron density matrix element and $H_{\mu\nu}$, the one-electron energy term which contains the electron-nucleus attractive energy and resonance energy terms. The SCF procedure does not directly affect the nuclear repulsion energy due to its independency of Fock matrix. For this reason, geometry optimization may not be always necessary for all cases. Our experience with the MINDO/3 indicates that various electronic properties that are evaluated directly from $P_{\mu\nu}$ and $F_{\mu\nu}$ in (2) are not greatly perturbed unless changes in geometric configurations are too excessive; among the less sensitive physical properties are atomic distributions, dipole moments, ionization potentials (in the unit of eV) and nuclear magnetic coupling constants. However, it is clear that such properties (of chemical importance) as equilibrium geometry, heat of formation (in the unit of kcal/mole), reaction path and normal vibrational frequency will be inaccurate without the geometry optimization process with the MINDO/3. Since we are largely interested in the study of the electronic structures of various molecules in isoalloxazine series, we will tolerate the use of fixed geometries for the Hartree–Fock SCF procedure involved with the MINDO/3.

Molecular geometries (bond distances, bond angles, and dihedral angles) were transformed to Cartesian coordinates [41] directly from fractional coordinates published for X-ray crystallography studies. The bond distances, bond angles, and dihedral angles were calculated using common vector methods. The electronic properties of oxidized lumiflavins were calculated for three different fixed geometries: *A* [42], *B* [43], and *C* [44]. The calculations for 10-methylisoalloxazine and isoalloxazine used a fourth geometry [45]. In some cases other geometries were attempted, but they failed to converge even after application of dampening techniques. The bond distances, bond angles, and dihedral angle of H3 in *D* were substituted for the 3-methyl group on *A*. While *A* and *D* were taken from the study of neutral molecules, *B* and *C* are progressively more cationic in nature, being derived from the study of flavin complexes.

The neutral lumiflavin molecules have a basis set of 88 atomic orbitals from valence shell electrons (see Fig. 1 for atomic structure and numbering). There are then 88 eigenvectors in the MINDO/3 calculation for lumiflavin. 5-deazalumiflavin replaces N5 with C5 and adds an extra hydrogen atom while the three anionic states considered (negative charges at positions C8α, C7α, and N3) have one less 1*s* orbital. 10-methylisoalloxazine substitutes 2 hydrogens for the 2 methyl groups on the benzenoid ring of lumiflavin while isoalloxazine has an additional replacement of the methyl group at N10 by a hydrogen atom.

In general, the largest negative charge centers are predicted to be on O2α, O4α, N1 and N3, as is seen in Table 1. In the neutral lumiflavin and 10-methylisoalloxaine molecules, the negative charge is concentrated on the two carbonyl oxygen atoms and the N1 and N3 nitrogen atoms. The positive charge accumulates on the C2 and C4 and, to a lesser degree, on the C10α carbon atoms. The most

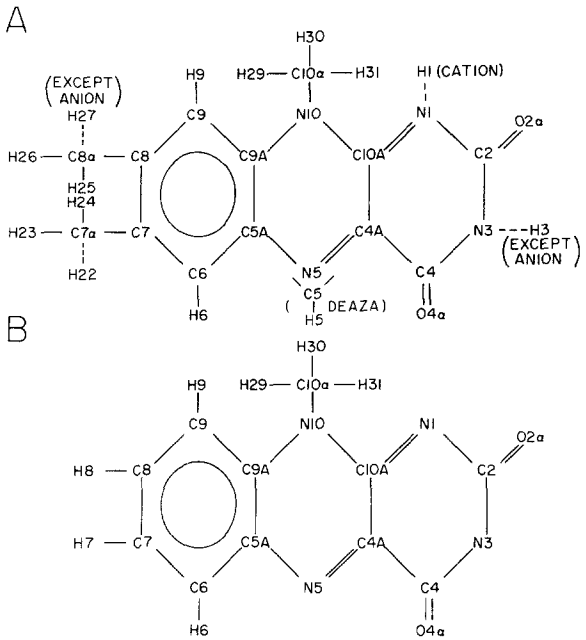

Fig. 1. Numbering and structure of (*A*) lumiflavin (*B*) 10-methylisoalloxazine.

acidic hydrogen is predicted to be H3. In the 5-deazalumiflavin molecule, the charge on C4*a* becomes noticeably negative, and the charge on C5 is quite positive (+0.1356) compared to the nearly neutral charge on N5 in all other computed neutral oxidized flavin molecules. The addition of a hydrogen at N1 is seen to substantially reduce the negative charges on N1 and N3 while H3 becomes acidic, as is seen in the table. As expected, removal of a hydrogen at the C8α C7α, or N3 positions increases the negative charge at these atomic sites with slight increases in negativity at the N1, O2, and O4 atoms. For the C8α anion, C4*a* becomes more negative and N5 and C9*a* increase in positive charge as shown in Table 1. In the C7α anion, the increase in negativity of C4*a* is about half that of the C8α anion and C9*a* becomes significantly more negative compared to the neutral molecules. In the case of the N3 anion, N5 is no longer nearly neutral but becomes significantly negative while C4*a* becomes almost neutral and C9*a* becomes more negative. The striking effect of excitation to the lowest singlet or triplet state is the partial transfer of negative charge from N1 and N3 to other atoms (for instance, C2 and C4*a*). For the oxidized flavin states, the net charge is distributed only on the pyrimidine ring, the negative charge on the heteroatoms, and the positive charge on the carbons. The notable exception is C4*a* which is slightly negative and becomes more negative upon ionization, excitation, or replacement of the N5 by C5. Removal of a hydrogen atom from any of the methyl groups tends to build up negative charge only on the adjacent α carbon; this ability would not be present in the isoalloxazine molecule.

Table 1. Net Atomic Charges[a]

Atom No.	$Lf_{ox}H^{A}$	$Lf_{ox}H^{B}$	$Lf_{ox}H^{C}$	$DzLf_{ox}H_2^{A}$	$Lf_{ox}H_2^{+C}$	$Lf_{ox}^{-A(C8\alpha)}$	$Lf_{ox}^{-A(C7\alpha)}$	$Lf_{ox}^{-A(N3)}$	$Lf_{ox}H^{TB}$	$Lf_{ox}H^{SA}$	$10MI_{ox}H^{D}$
N1	−0.4053	−0.4489	−0.4513	−0.4147	−0.2440	−0.4600	−0.4674	−0.4462	−0.3344	−0.3248	−0.4030
C2	+0.7043	+0.7158	+0.7074	+0.7085	+0.6770	+0.7042	+0.6998	+0.7326	+0.6894	+0.6809	+0.7050
N3	−0.3182	−0.2907	−0.2925	−0.3195	−0.2732	−0.3285	−0.3271	−0.5804	−0.2739	−0.2998	−0.3142
C4	+0.6258	+0.6290	+0.6232	+0.6366	+0.6197	+0.6364	+0.6227	+0.6272	+0.6219	+0.6227	+0.6244
C4*a*	−0.1023	−0.0733	−0.0591	−0.2467	−0.0811	−0.2240	−0.1194	−0.0486	−0.1200	−0.1598	−0.0992
N5	−0.0120	−0.0286	−0.0370	+0.1356	+0.0434	+0.0050	−0.0385	−0.1074	−0.0333	+0.0056	−0.0131
C5*a*	+0.0013	+0.0228	+0.0330	−0.0929	+0.0436	−0.1010	+0.0901	+0.0239	+0.0973	+0.0690	+0.0090
C6	+0.0073	+0.0217	−0.0049	+0.0223	+0.0045	+0.0171	−0.2115	−0.0239	−0.0609	−0.0685	+0.0207
C7	−0.0166	−0.0161	−0.0224	−0.0289	+0.0178	−0.0721	+0.1743	−0.0379	+0.0343	+0.0285	−0.0513
C8	+0.0579	+0.0562	+0.0668	+0.0725	+0.1041	+0.1880	−0.0094	+0.0153	−0.0030	+0.0028	+0.0421
C9	−0.0961	−0.0609	−0.0811	−0.1112	−0.0767	−0.2640	−0.0697	−0.1008	−0.0407	−0.0732	−0.0752
C9*a*	+0.1026	+0.0781	+0.0649	+0.1466	+0.0701	+0.1609	−0.0310	+0.0880	+0.0503	+0.0752	+0.1028
N10	−0.0352	−0.0011	+0.0165	−0.0578	+0.0655	−0.0483	+0.0185	−0.0705	−0.0295	−0.0591	−0.0493
C10*a*	+0.2672	+0.2444	+0.2176	+0.3143	+0.2026	+0.2899	+0.2384	+0.2342	+0.2387	+0.2717	+0.2644
C7α	+0.0066	+0.0392	+0.0215	+0.0072	+0.108	+0.0280	−0.4974	+0.106	+0.0314	−0.0008	—

C8α	−0.0002	+0.0274	−0.0589	−0.0033	−0.0475	−0.4088	+0.0201	+0.0083	+0.0416	+0.0123	—
C10α	+0.0841	+0.0900	−0.0366	+0.0919	−0.0102	+0.0820	+0.0703	+0.0799	+0.1105	+0.1044	+0.0627
O2α	−0.5671	−0.5729	−0.5831	−0.5724	−0.5016	−0.6373	−0.6335	−0.6541	−0.5733	−0.5725	−0.5709
O4α	−0.5677	−0.5690	−0.5713	−0.5896	−0.4839	−0.6321	−0.6113	−0.6650	−0.5641	−0.5667	−0.5693
H3	+0.1385	+0.1047	+0.1180	+0.1347	+0.1777	+0.1075	+0.1107	—	+0.1170	+0.1454	+0.1382
H6	+0.0265	+0.0082	+0.0346	+0.0163	+0.0565	−0.0105	+0.0243	+0.0123	+0.0134	+0.0329	+0.0323
H22	+0.0003	−0.0031	−0.0211	−0.0007	+0.0041	−0.0173	−0.0011	−0.0184	−0.0020	+0.0019	—
H23	+0.0148	−0.0064	−0.0068	+0.0139	+0.0207	−0.0018	+0.0074	−0.0054	−0.0070	+0.0160	—
H24	+0.0030	−0.0012	+0.0317	+0.0018	+0.0457	−0.0332	—	−0.0114	+0.0003	+0.0031	—
H25	+0.0040	+0.0068	−0.0487	+0.0052	−0.0329	+0.0072	−0.0095	−0.0182	−0.0002	−0.0025	—
H26	+0.0096	−0.0060	+0.0665	+0.0107	+0.0862	+0.0014	−0.0054	−0.0118	−0.0120	+0.0038	—
H27	+0.0123	−0.0007	+0.0683	+0.0129	+0.0773	—	−0.0276	−0.0025	−0.0083	+0.0077	—
H9	+0.0282	−0.0085	+0.0242	+0.0302	+0.0399	+0.0269	−0.0105	+0.0111	−0.0140	+0.0265	+0.0219
H29	−0.0032	+0.0253	+0.1028	−0.0050	+0.1002	−0.0072	−0.0060	−0.0281	+0.0143	−0.0046	+0.0488
H30	−0.0126	+0.0089	+0.0060	−0.0142	+0.0041	−0.0283	−0.0265	−0.0451	+0.0120	−0.0117	−0.0064
H31	+0.0419	+0.0080	+0.0699	+0.0422	+0.1005	+0.0199	+0.0265	+0.0325	+0.0042	+0.0336	+0.0269
H1	—	—	—	—	+0.1785	—	—	—	—	—	—
H7	—	—	—	—	—	—	—	—	—	—	+0.0398
H8	—	—	—	—	—	—	—	—	—	—	+0.0129
H5	—	—	—	+0.0536	—	—	—	—	—	—	—

[a] Lf = oxidized lumiflavin; $10MI_{ox}$ = oxidized 10-methylisoalloxazine; H, H_2 = ionizable protons; *A*, *B*, *C*, *D* = geometry used; *T*, *S* = triplet and singlet states; +, − = charge; C8α, C7α, N3 = position from which proton is removed.

A correlation exists between the net charge and the *pKa* values for nitrogen atoms in heterocyclics [46]. As is exhibited in Table 1, the net charge densities for the four nitrogen atoms of "neutral oxidized" lumiflavin show N1 to be a very basic "pyridinyl" nitrogen, N3, to be a very basic "pyrrolic" nitrogen, and the N10 and N5 nitrogens to be essentially neutral. In both the singlet and triplet excited states, the basicity of the N1 decreases approximately 0.1 charge units while the N3 decreases about 0.02. This would predict a lower *pKa* for the cation-to-neutral equilibrium in the excited states compared to the ground state, if we assume the nitrogens are the atoms involved in protonation. EPR results indicate that the triplet has a higher cation to neutral *pKa* than both the ground and singlet states [47]. A possible explanation is that the oxygens, which have the highest charge density of all the atoms in lumiflavin, may become protonated in the triplet state.

MINDO/3 has been shown to yield nuclear magnetic coupling constants in fair agreement with experimental values [48]. Chemical shifts are correlated with atomic charge densities. Better correlation with experiments will be a likelihood when total charge density including σ electrons is used rather than the π electron density [49]. Charge densities from CNDO or PPP type calculations did not even give fair qualitative predictions. This was expected as in the prediction of unpaired electron densities, these approximations are too crude to yield a desirable accuracy in the spin polarization contribution to the unpaired electron density.

The neutral atomic charge densities predicted in our present MINDO/3 study have been compared with experimental NMR data for ^{2}H and ^{13}C labelled 3,10-dimethylisoalloxazine [50, 51]. For the ^{2}H labelled molecule, the chemical shifts calculated from the MINDO/3 charge densities correlate, within experimental error, to the observed values, while other calculations such as CNDO/2 and PPP did not correlate at all. Comparing the chemical shifts for the C6, C7, C8, and C9 atoms, the observed data indicated that the C8 atom is less shielded from the magnetic field than the C7 atom and that the C6 atom is at very low field while the C9 atom is shifted quite far upfield. The C4*a* atom is at much higher field than the measured resonance position of C4*a* at high field, and C10*a* at low field shows good correlation with the relative charges. In short, the observed chemical shifts are in good correlation with the charge distributions shown in Table 1, but the exact magnitude agreement remains to be improved.

The total dipole moment (μ) is a directional quantity (from negative charge center to positive charge center) which measures the polarity of charge distribution. In the neutral flavin molecules, the computed dipole vector is directed toward the benzenoid ring at an angle of 105° counterclockwise from an axis drawn approximately between the N1 nucleus and the C4*a* nucleus as shown in Fig. 2. The direction is generally in good agreement with the results obtained by CNDO methods for isoalloxazine [53]. The addition of a methyl group is predicted to have little effect on the magnitude and direction of the dipole moment, as is easily seen from Table 2 and Fig. 2.

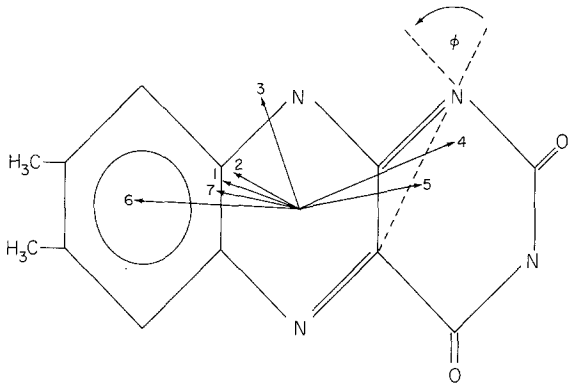

Fig. 2. Total dipole moments of oxidized flavins (1) lumiflavin and 10-methylisoalloxazine (2) lumiflavin singlet and lumiflavin triplet (3) N3 lumiflavin anion (4) C7α lumiflavin anion (5) C8α lumiflavin anion (6) N1-lumiflavin cation (7) 5-deazalumiflavin.

Table 2. Dipole Moments (Debyes)

	Total dipole	Total x	Total y	Total z	$\theta(x, y)$[a]
$Lf_{ox}H^A$	8.681	−8.384	2.248	−0.070	105°
$Lf_{ox}H^B$	9.466	−9.126	2.499	0.273	105°
$Lf_{ox}H^C$	9.962	−8.932	3.112	3.126	109°
$Lf_{ox}H_2^{+C}$	17.158	−14.058	8.476	4.992	121°
$Lf_{ox}^{-A(C8\alpha)}$	13.125	6.882	−11.157	−0.651	328°
$Lf_{ox}^{-A(C7\alpha)}$	18.257	6.130	−17.187	−0.595	340°
$Lf_{ox}^{-A(N3)}$	12.582	−10.455	−6.997	−0.156	34°
$Lf_{ox}H^{SA}$	7.407	−7.277	1.383	0.071	101°
$Lf_{ox}H^{TB}$	7.537	−7.473	0.964	0.187	97°
$DzLf_{ox}H_2^A$	8.962	−8.097	3.841	0.042	115°
$10MI_{ox}H^D$	8.392	−8.093	2.150	0.553	105°
$ISOA_{ox}H^D$ [b]	8.163	−7.769	2.472	0.414	108°

[a] The angle is measured counterclockwise from the N1–C4a axis.
[b] ISOA = isoalloxazine.

MINDO/3 predicts that 5-deazalumiflavin has a dipole moment comparable in magnitude to the lumiflavin molecules (A, B, and C) of three different geometries. Its dipole moment is rotated about 10° counterclockwise with respect to the other neutral flavins. Excitation is seen to reduce the magnitude of the dipole moment and rotates it clockwise 4° to 6° towards the short molecular axis. On the other hand, CNDO results indicated a slight rotation in the opposite direction for the triplet of isoalloxazine [53]. Contrary to our results, others reported that the excited states showed more polarity than the neutral states [3]. A decrease in the dipole moment upon excitation could suggest a partial charge transfer from an n-orbital to π^*-orbital. Certainly the $n \rightarrow \pi^*$ transition is known to reduce the magnitude of the dipole moment [54]. However, our result (Fig. 3)

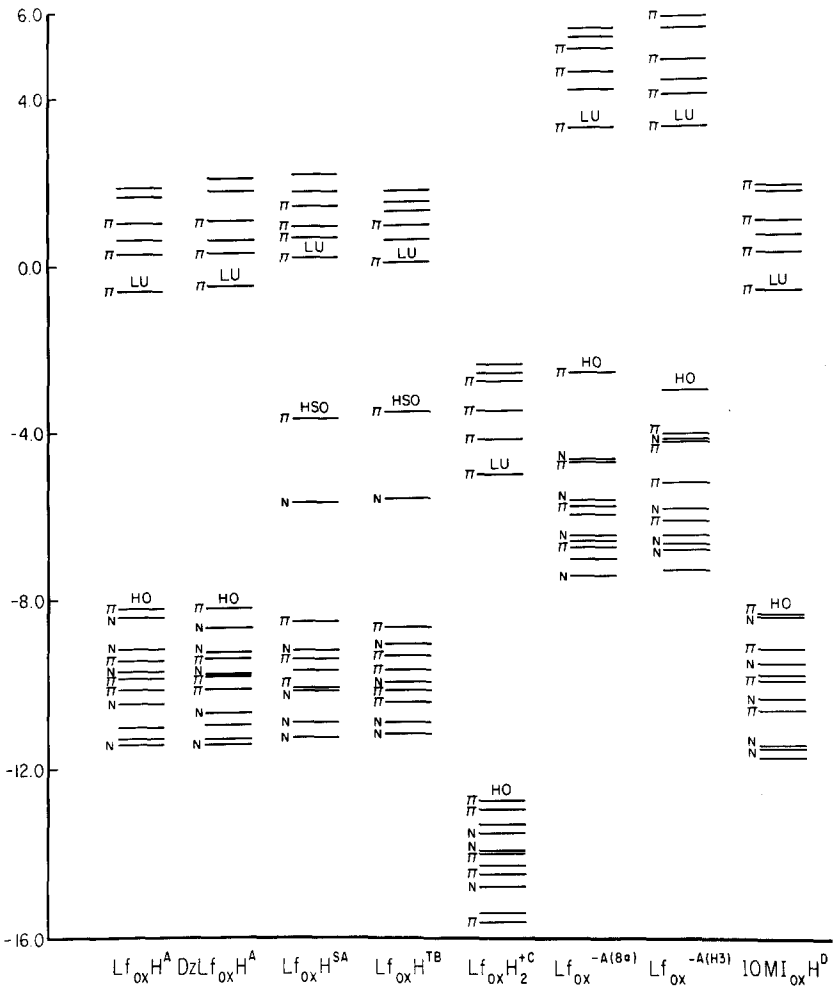

Fig. 3. A set of the highest filled and lowest unfilled energy levels (eV), HO = Highest Occupied MO, LU = Lowest Unfilled MO, HSO = Highest Singly Occupied MO, $\pi = \pi$ oribtal, N = Non-bonding orbital.

shows a $\pi \rightarrow \pi^*$ transition. Thus, the calculations indicate that a $\pi \rightarrow \pi^*$ transition may also contribute to the reduction of the dipole moment.

Curiously, the predicted N1 cation dipole moment increases to almost twice that of the neutral lumiflavin. The C7α anion dipole moment increases one and one-half times in magnitude over the C8α anion, but in the opposite direction to the dipole moment of the neutral molecule. Protonation rotates the dipole moment about 16° clockwise. Formation of the anionic states reverse the direction of the charge centers. This possibly reflects either the inability of the pyrimidine ring to absorb extra negative charge or the inability of MINDO/3 to redistribute charges via orbital overlap.

Grabe [2] has reported a value of 8.92 Debyes for the μ^{π} of lumiflavin from a PPP calculation. This is in relative agreement with our result of 8.68 Debyes. A CNDO calculation yielded a dipole moment of 5.5 Debyes for isoalloxazine [3], compared to our 8.2 Debyes.

The computed π orbitals are largely from the p_z AO population. Six orbitals come from the benzenoid ring, six from the primidinoid ring, one from each oxygen (2), and an extra π orbital from both the N3 and the N10 pyrrolic nitrogens. Six non-bonding orbitals (five for the cation) are assumed, based on the assumption of a non-bonding electron pair donated by both the N1 and N5 "pyridinyl" nitrogens (in the cation the N1 non-bonding electron pair converts to a σ orbital) and two non-bonding electron pairs donated by each carbonyl oxygen.

By this scheme, the HOMO in the neutral species is found to be a π orbital, as is the LUMO. In contrast, the second highest occupied MO in the neutral species and the second singly occupied orbital in the excited states are predicted to be n orbitals. If we look at the AO content of the highest orbitals in Table 3, we see that in the HOMO and LUMO of the ground states (lumiflavin, 5-deazalumiflavin, and 10-methylisoalloxazine) over 95% of the electron population comes from the p_z AO, while in the second HOMO of the neutral ground

Table 3. AO Contributions to LUMOS and HOMOS

		S	P_x	P_y	P_z
$Lf_{ox}H^A$	LUMO[a]	0.073	0.019	0.048	1.89
	HOMO[b]	0.065	0.018	0.064	1.85
	2*d* HOMO	0.243	0.455	1.23	0.076
$Lf_{ox}H^{SA}$	HSOMO[c]	0.063	0.022	0.048	1.87
	2*d* HSOMO	0.258	0.377	1.31	0.052
	HOMO	0.069	0.042	0.010	1.88
$Lf_{ox}H^B$	LUMO	0.065	0.016	0.060	1.86
	HOMO	0.091	0.068	0.569	1.27
	2*d* HOMO	0.208	0.315	0.835	0.643
$Lf_{ox}H^{SB}$	HSOMO	0.066	0.019	0.073	1.84
	2*d* HSOMO	0.218	0.223	1.37	0.191
	HOMO	0.089	0.078	0.084	1.75
$DzLf_{ox}H_2^A$	LOMO	0.070	0.017	0.044	1.87
	HOMO	0.061	0.026	0.031	1.88
	2*d* HOMO	0.219	0.513	1.25	0.015
$10MI_{ox}H^D$	LUMO	0.061	0.031	0.049	1.86
	HOMO	0.032	0.061	0.014	1.89
	2*d* HOMO	0.244	0.465	1.23	0.056
Pyridine	LUMO	0.001	0.000	0.000	1.99
	HOMO	0.448	0.434	1.12	0.001
	2*d* HOMO	0.000	0.001	0.000	1.99

[a] LUMO = Lowest Unoccupied MO.
[b] HOMO = Highest Occupied MO.
[c] HSOMO = Highest Singly Occupied MO.

states and in the second singly occupied orbitals of the excited states 62 to 67% of the electron population comes from the p_y AO. Pyridine is also shown for comparison where the highest orbital, an n orbital, has mostly p_y AO content. The results of labelling these orbitals are uniformly consistent for the highest orbitals among the three geometries of neutral oxidized lumiflavin, 5-deazalumiflavin, and neutral oxidized 2-methylisoalloxazine (Fig. 3). The HOMO and the LUMO are π orbitals and the second highest occupied MO is an n orbital. It is interesting to note that while the C8α and C7α anions retain this structure, the N3 anion and the N1 cation do not. The HOMO of the N3 anion is labeled as a σ orbital.

The calculated molecular orbital energy levels corresponding to the three different lumiflavin geometries vary generally by only 0.1 eV. In a large molecule like lumiflavin, the MINDO/3 MO structure is quite insensitive to small differences in geometry (as was previously discussed). With the increasing availability of X-ray crystallography data for biological molecules, this makes MINDO/3 an attractive theoretical tool in biology.

The two highest occupied orbitals in the neutral molecules show quasidegeneracy with the energy spacings of 0.17 eV for lumiflavin and of 0.03 eV for 10-methylisoalloxazine. The predicted highest orbital is a π orbital and the second highest is a non-bonding orbital for these molecules. Although in many hetero-conjugated molecules a lone pair orbital is the highest orbital, in pyridine a non-bonding orbital and a π orbital are quasi-generate and the first $n \rightarrow \pi^*$ transition has the same wavelength as the first $\pi \rightarrow \pi^*$ transition and is hidden by it [52]. The MINDO/3 calculated orbital energies suggest that a similar phenomenon may occur with flavin molecules. The computed gap between the two singly occupied orbitals in the excited states is around 2.0 eV, and the singlet and triplet interval is less than 0.01 eV, characteristic of an $n \rightarrow \pi^*$ transition. However, the magnitudes of these values should be qualitatively correct, even though besides the exclusion of configuration interaction terms, the geometries of the excited state molecules were taken to be the same as the ground state molecules in the present MINDO/3 study.

Recently, from their molecular luminescence measurements of some isoall-oxazines in apolar solvents, Eweg et al. [55] found close agreement with our predicted electronic transitions. For instance, the observed wave number range of 21 300–21 500 cm^{-1} for the S_0–S_1 transition [55] compares well with our previously unpublished value of 21 700 cm^{-1} (for further details, see [55]).

The energy difference (0.17 eV) between the LUMO of lumiflavin and the LUMO of 5-deazalumiflavin suggests that both the oxidized 5-deazalumiflavin and lumiflavin are almost equally electrophilic. Interestingly, the HOMOS of these molecules are predicted to have the same energy. The two electron redox potential of 5-deazariboflavin (ca. −0.31 V) is reported to be lower than that of riboflavin (ca. −0.21 V) [56].

Table 4 shows our first calculation to give an estimate for the total energy of a flavin molecule. From these energies, we can calculate the proton ionization

Table 4. Energies

	Total energy eV	T.E.E.[a] eV	C.C.R.E.[b] eV	I.P.[c] eV	E.A.[d] eV	Gr-Ex gap[e] eV
$Lf_{ox}H^{A}$	−3211.43	−20 323.11	+17 111.68	8.19	0.62	
$Lf_{ox}H^{B}$	−3214.95	−20 256.42	+17 041.47	8.11	0.71	
$Lf_{ox}H^{C}$	−3200.25	−20 353.90	+17 153.66	8.07	0.75	
$Lf_{ox}H_2^{+C}$	−3205.67	−20 762.73	+17 557.06	12.69	5.01	
$Lf_{ox}^{-A(C8\alpha)}$	−3198.03	−19 981.67	+16 783.64	2.61	−3.26	
$Lf_{ox}^{-A(C7\alpha)}$	−3197.56	−20 022.80	+16 825.24	2.14	−2.85	
$Lf_{ox}^{-A(N3)}$	−3197.15	−19 965.27	+16 768.12	3.05	−3.26	
$Lf_{ox}H^{SA}$	−3209.44	−20 321.12	+17 111.68	3.54	−0.21	1.99
$Lf_{ox}H^{SB}$	−3213.03	−20 254.50	+17 041.47	3.53	−0.06	1.92
$Lf_{ox}H^{TB}$	−3213.42	−20 254.89	+17 041.47	3.53	−0.06	1.54
$DzLf_{ox}H_2^{A}$	−3156.65	−20 264.57	+17 107.92	8.20	0.46	
$10MI_{ox}H^{D}$	−2899.76	−16 861.01	+13 961.25	8.34	0.67	
$ISOA_{ox}H^{D}$	−2746.39	−14 928.83	+12 182.44	8.49	0.75	

[a] T.E.E. = Total Electronic Energy.
[b] C.C.R.E. = Core-Core Repulsion Energy.
[c] I.P. = Ionization Potential.
[d] E.A. = Electron Affinity.
[e] Gr-Ex Gap = difference in total energies between the ground state and the excited state for the same geometry.

energies (energy required to remove a proton) of lumiflavin: 13.40 eV at C8α, 13.77 eV at C7α, and 14.28 eV at N3. On the other hand, addition of a proton at N1 releases 5.42 eV. Replacement of the N5 by C5 in 5-deazalumiflavin requires 55.08 eV.

The computed ionization potentials that appear in Table 4 are essentially from the canonical molecular orbitals by Koopman's theorem. The electron affinity for lumiflavin (0.62 eV) (the negative of the LUMO) compares with the values of 0.55 eV calculated for the difference in total energy between the oxidized neutral lumiflavin molecule and the semiquinone anion. Previous π calculations of flavins reported much higher electron affinities for neutral oxidized flavin molecules [57]. However it is to be added that in general, such application of Koopman's theorem on a virtual orbital may not be accurate, particularly with semi-empirical methods which use only valence electrons. Orbital energies by NDO methods may contain systematic and random errors up to ±2 eV [58]. Thus, a serious comparison of absolute magnitudes should be avoided. For this reason, this work may yield good correlation with measurements, rather than numerical agreement with them.

The energy of the first singlet is calculated as lying 1.99 eV (623 nm) higher than the ground state, and the triplet transition is 1.54 eV (805 nm) higher. Both of these energy values are lower than the experimental values measured in solution (2.35–2.48 eV [59] for the singlet and 1.91–2.07 eV [60] for the triplet). Our theoretical values should not agree well in absolute magnitude since the geometric structure in solution is in bent conformation compared to the planar

structure we chose. The gas phase structure is now believed to be planar [61]. However, it is interesting to note that the computed energy ordering is in perfect agreement with the experiment. However, Eweg et al. [61] reported excellent agreement for their photoelectric spectra with our computed π-orbital energies (Table 3). In addition, they found that our results of the orbital crowding in the -8 to -12 eV energy range shown in Fig. 3 were in excellent agreement with their observed structureless photoelectron spectrum.

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