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The Role of Aromaticity in the Planarity of Lumiflavin

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Ab initio MP2/6-31G(d,p) and density functional theory B3LYP/6-31G(d,p) calculations were performed to investigate the molecular structure of the active part of flavins in the oxidized and reduced forms, using lumiflavin as a model compound. The possible aromatic character of these systems was explored by using the following aromaticity indexes: nucleus-independent chemical shifts, the anisotropy of the magnetic susceptibility, the Bird index, and natural bond orbital analysis. To provide further insight, calculations on the 2+ charged species were also carried out. Both the MP2 and B3LYP computations predict a planar conformation for the oxidized form and a bent structure for the reduced form, in agreement with previous experience. For both the oxidized and reduced states, ring A is found to be the most aromatic, as expected. The calculations suggest that the folding in the reduced form is mainly a result of electronic preferences rather than steric hindrance.

1. Introduction

The physical and chemical properties of flavin derivatives have been of considerable experimental and theoretical interest due to the important role that flavins play as biological redox reagents.[1−4] The flavin redox system is commonly described by three different oxidation states: oxidized (quinone), radical (semiquinone), and reduced (hydroquinone) forms. The active part of flavins is a 7,8-dimethylisoalloxazine substituted at nitrogen 10 (see Figure 1). The electronic structure of the flavin (and particularly the planarity of the isoalloxazine ring) together with the protein environment are the main factors that govern the flavin reactivity and, in particular, the redox potential of flavoproteins.[5−7]

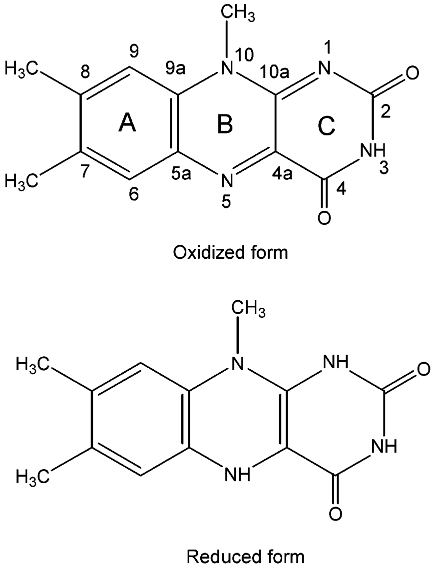

FIGURE 1. Oxidized and reduced forms of lumiflavin. The usual valence-bond representations are depicted. The atom numbering is shown in the oxidized structure.

The elucidation of the isoalloxazine molecular structure has attracted the interest of experimentalists and theoreticians. Crystal structure determinations on substituted isoalloxazines show that the oxidized form is planar,[8−11] although NMR spectroscopy suggests that, in aprotic solvents, the N(10) atom is located out of plane to a certain extent.[12] The crystal packing forces could play a significant role in the conformations of crystallized

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flavins. On the other hand, the X-ray diffraction studies on reduced species show that the isoalloxazine ring is significantly puckered along the N(5)−N(10) axis.[13,14] However, NMR investigations led Moonen et al.[12] to suggest that the reduced isoalloxazine is only slightly bent in apolar solvents and that the bend is decreased in protic solvents of high dielectric constants due to the formation of hydrogen bonds. In addition, they concluded that the solution structure of reduced flavin is mainly governed by steric hindrance and hydrogen bonds. The barrier for the ring inversion is estimated to be less than 5 kcal/mol.[12] The experimental information concerning the molecular structure of the radical form is scarce due to its instability. EPR and ENDOR spectroscopy studies on flavin radicals suggest that the isoalloxazine ring for this form may be rather planar.[16,17]

Several theoretical studies have been performed to provide additional insight into the molecular structure of simple isoalloxazines. The first investigations involved semiempirical methods and single-point ab initio calculations.[18−26] With the development of powerful computers in the 1990s, more rigorous calculations on these systems became feasible.[27−37] The three oxidation forms of lumiflavin, the most widely used model compound for flavin, were explored by Hartree−Fock (HF) calculations with the standard 3-21G and 6-31G(d) basis sets.[27−29] More recently, the simplest model compounds isoalloxazine and 10-methylisoalloxazine were investigated at the B3LYP/6-31G(d) and MP2/6-31G(d,p)//HF/6-31G(d) levels of accuracy, respectively.[30,31] All of these calculations predict that the isoalloxazine ring is planar in the oxidized form, folded in the reduced form, and planar or very close to planar in the radical form, which is in general agreement with the experimental findings.

Despite the numerous studies directed at determining the molecular structure of the isoalloxazine system, a question still remains as to whether the planarity vs nonplanarity of the flavin is a consequence of steric and/or electronic effects. The answer to this question might be related to the possible aromatic character of the isoalloxazine ring, as suggested sometimes. Confusion around this topic appeared in the earlier studies. Thus, Hall et al.[22,38] stated that the oxidized form should be nonaromatic, in contrast to what has usually been accepted. Similarly, Tauscher at al.[20] considered the reduced form to be antiaromatic, but later Hall et al.[38] stated that this is not so. Some insight into this issue was provided by HF/3-21G and MINDO/3 calculations on 2+ and 2− charged species for both the oxidized and reduced forms of lumiflavin.[27] For the charged oxidized form, both the HF and MINDO/3 calculations predict a folded structure. Since the neutral species is planar, this suggests that the neutral oxidized species may have some aromatic character. For the reduced form, the MINDO/3 calculations on the charged species predict structures essentially planar, indicating that the neutral reduced form is not aromatic. The HF/3-21G calculations afford a bent structure for both the neutral and 2+ species, suggesting that the neutral reduced form is not antiaromatic. However, electron correlation, not considered in that study,[27] is known to be important for delocalized systems,[39] and so the above results should be taken with care.

In this study, we present ab initio MP2 and density functional theory (DFT) calculations, using the standard 6-31G(d,p) basis set, on the oxidized and reduced states of lumiflavin in order to provide further insight into the molecular structure properties and the possible aromatic character of these species. To this end, we performed calculations on the neutral and 2+ charged species and applied the following known criteria for aromaticity: the "nucleus-independent chemical shifts" (NICSs),[40] the anisotropy of the magnetic susceptibility (χ_{anis}),[41,42] and the unified Bird index (I_A).[43] Additionally, the MP2 and DFT electron densities were investigated in the light of the natural bond orbital (NBO) method.[44−46]

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2. Computational Methods

The oxidized and reduced forms (neutral and 2+ charged species) of lumiflavin were optimized by DFT calculations, employing the B3LYP approach and the standard 6-31G(d,p) basis set. This DFT method combines Becke's three-parameter nonlocal hybrid exchange potential[47] with the nonlocal correlation functional of Lee, Yang, and Parr.[48] Optimizations were also carried out at the Møller−Plesset perturbation level with the inclusion of energy corrections through second-order (MP2). The calculations were performed with the GAUSSIAN 98 program.[49] The nomenclature employed to name the structures studied in this work resembles that used in ref 27: O_f for the oxidized form and R_f for the reduced one, where the subscript f indicates that the structure is obtained from full geometry optimization. For the full-optimized conformations, B3LYP/6-31G(d,p) frequency calculations were performed to characterize them as minima or as saddle points.

Partial optimizations were performed to explore the energetics of ring folding about the N(5)−N(10) axis. In those cases in which the full optimization led to a folded conformation, a partial optimization was carried out, constraining all the atoms other than hydrogen to be in a plane. When the full optimization led to a planar structure, a partial optimization was performed, fixing several dihedral angles so as to obtain a fold angle of ca. 20°.[50] The fold angle is a geometric parameter frequently used to describe approximately the molecular shape of the isoalloxazine ring and is defined as the angle between normals to the least squares planes: N(1)−C(2)−N(3)−C(4)−C(4a)−C(10a)−N(5)−N(10) and C(6)−C(7)−C(8)−C(9)−C(9a)−N(5)−N(10). The subscript c was used to indicate that geometric constrains were established in the calculation. For example, the full-optimized structure for the oxidized form (O_f) is predicted to be planar; therefore, a bent structure with a fold angle of ca. 20° was optimized under geometric constrains (O_c).

Three known criteria for aromaticity were employed in this study. Two of them are magnetic criteria: NICSs[40] and the anisotropy of the magnetic susceptibility (χ_{anis}).[41,42] The other is a geometry-based criterion: the Bird index in its unified form (I_A).[43] NICS values were evaluated by using the gauge invariant atomic orbital[51] (GIAO) approach at the GIAO-SCF/6-31+G(d) level with the MP2/6-31G(d,p) geometries. This level of accuracy has proven to be reliable for exploring aromaticity.[40] These calculations were performed at the ring critical points (3, +1)[52] using the wave function topological analysis from Bader's "theory of atoms in molecules",[53] as implemented in the AIMPAC suite of programs.[54] The anisotropy of the magnetic susceptibility was computed with the "individual gauges for atoms in molecules" (IGAIM) method,[55] which is a slight variation on the Continuous Set of Gauge Transformations (CSGT) method.[55,56] As for the NICS values, the evaluation of magnetic susceptibilities involved HF/6-31+G(d) computations. Finally, electron densities were analyzed with the NBO method,[44−46] implemented in GAUSSIAN 98, using the MP2/6-31G(d,p) and B3LYP/6-31G(d,p) electron densities.

TABLE 1. Relative Energies (in kcal/mol) for the Conformations Studied in This Work

	shape	B3LYP	MP2
O_f	planar	0.0	0.0
O_c	bent	5.2	4.4
R_f	bent	0.0 (156.0°, 154.2°)[a]	0.0 (150.4°, 150.5°)[a]
R_c	planar	2.3	6.9
O_f^{2+}	planar	0.0	0.0
O_c2+	bent	4.7	4.9
R_f^{2+}	~planar	0.0 (175.0°, 178.7°)[a]	0.0 (174.3°, 178.4°)[a]
R_c2+	bent	4.2	4.2

[a] The first number in parentheses is the mean value for the torsion angles (absolute values) N(1)−C(10a)−N(10)−C(9a) and C(9)−C(9a)−N(10)−C(10a). The second value is for C(4)−C(4a)−N(5)−C(5a) and C(4a)−N(5)−C(5a)−C(6).

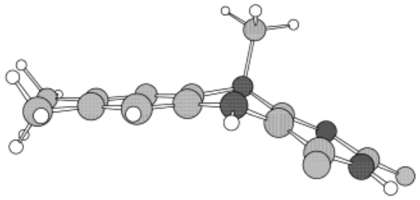

FIGURE 2. The (neutral) reduced form of lumiflavin optimized at the MP2/6-31G(d,p) level.

3. Results and Discussion

The relative energies for the species investigated in this study are collected in Table 1. The B3LYP/6-31G(d,p) frequency calculations showed that all the full-optimized structures investigated here are minima. The MP2 and B3LYP full optimizations afford planar structures for the oxidized form (O_f), in agreement with previous ab initio studies performed at the HF level of calculation.[27,28] The relative energies for O_c, a constrained, bent conformation with a fold angle of ca. 20°,[50] are predicted to be 4.4 and 5.2 kcal/mol with the MP2 and B3LYP methods, respectively. These relatively small values suggest that the planarity of the oxidized form may be influenced upon binding to apoflavoproteins.

The results for the reduced form (R_f) indicate that the preferred conformation is bent, in agreement with previous experience. Rings A and C are planar, and ring B is found to be in a boatlike conformation, as depicted in Figure 2. A fold angle of 35° was observed in a crystallographic structure of an analogue of reduced flavin.[13]

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The fold angles predicted by the MP2 and B3LYP methods are somewhat lower: 30 and 25°, respectively. The presence of bulky groups and packing forces, however, may influence the value in the crystal structure. Both the MP2 and B3LYP calculations predict that the N(5) center is pyramidal, although less pyramidal than a tetrahedral nitrogen center, with the attached hydrogen in an equatorial orientation. However, the results for the N(10) atom show a substantial discrepancy. The MP2 calculations predict a pyramidal geometry around N(10) and an axial orientation for the methyl group. By contrast, the B3LYP calculations show an essentially planar geometry around N(10). It has been found for other systems that B3LYP tends to lead to more planar structures compared to MP2; for example, amino groups of guanine are less pyramidal[58] and also biphenyl and related aromatic compounds with a single torsional degree of freedom are more planar and have lower torsional barriers at the DFT level.

Partial optimizations were performed to give an estimate of the barrier for ring inversion in the reduced form. The results are shown in Table 1. If we consider the relative energy of the R_c conformation as an approximation of the inversion barrier (more specifically, an upper limit since ring flattening and nitrogen center inversion could take place in separate steps), the values predicted by MP2 and B3LYP are 6.9 and 2.3 kcal/mol, respectively. The higher value obtained by MP2 is related with the higher fold angle and the degree of pyramidal character at N(10), as compared with the B3LYP geometry. The MP2 value is similar to that obtained at the HF/6-31G(d) level of accuracy (6.4 kcal/mol),[28] and the B3LYP result is similar to that calculated at the HF/3-21G level (2.4 kcal/mol).[27] NMR investigations[12] indicate that the barrier for ring inversion is less than 5 kcal/mol. Therefore, our results are in agreement with the experimental estimate and corroborate that the conformation of reduced flavin in flavoenzymes may be easily modulated by the protein environment.

To assess the extent to which the folding in the reduced form of lumiflavin comes from steric hindrance, we performed additional calculations on 7,8-dimethylisoalloxazine, that is, the methyl group at N(10) was replaced by H. The MP2 and DFT calculations afforded fold angles of about 23 and 17°, respectively, and barriers for ring inversion of ca. 4.2 and 1.1 kcal/mol, respectively. The fold angle and the barrier for ring inversion increase somewhat upon methyl substitution at N(10), a trend predicted previously by Meyer[30] in analogous compounds. Although these calculations show that steric hindrance has a certain role in the conformation of the reduced form of lumiflavin, even more important is the influence of the electronic effect. In fact, the optimized conformation for the 2+ charged reduced species (R_f^{2+}) is predicted to be almost planar at both the MP2 and B3LYP levels of theory (see Table 1), suggesting that to a large extent the folding in the neutral reduced species is a consequence of electronic preferences. This appears to be in contrast with the conclusion of Moonen et al.[12] that, in solution, steric hindrance plays a fundamental role in the conformation of reduced flavin. A source of disagreement may come from the effects of the solvent, which may influence the conformations of flavins.[30,31]

As indicated in the Introduction, there has been so much discussion and confusion in the literature regarding the aromaticity of flavins[9,11,12,20,22,27,38] that we thought it to be of interest to pay special attention to this issue. It is well known that there are several criteria in the literature for the classification of aromaticity,[40,59−66] and so no universal definition exists. In this study, to explore the possible aromatic properties of the oxidized and reduced forms of lumiflavin, we used the following criteria for aromaticity: NICSs,[40] the anisotropy of magnetic susceptibility,[41,42] the unified Bird index,[43] and the natural bond orbital method.[44−46] On the other hand, if aromaticity is considered as a special stability due to the conjugation of a π-system in a ring,[67] a change in the π-electron population (by removing or adding electrons) could affect the structure of the molecule significantly. If so, any differences in structure between the neutral and charged forms are the result of purely electronic preferences. Accordingly, to provide additional insight, we have also investigated 2+ charged species. To a large extent, the study of these charged species was inspired in the famous $4n + 2$ Hückel rule, which is quite often applied to polycyclic compounds, although its use is strictly justified for monocyclic systems only.[68]

As noted previously, the 2+ charged species for the reduced form is predicted to be almost planar with the two methods applied (MP2 and B3LYP), with the most important deviations involving atoms of ring B. In addition, the calculations on a constrained, bent structure R_c^{2+} give an energy 4.2 kcal/mol higher than R_f^{2+}. At first glance, our results, which are in agreement with previous MINDO/3 calculations but not with the HF/3-21G results (predicting a bent structure for R_f^{2+}),[27] seem to suggest that the neutral reduced form is not aromatic, or more rigorously, that ring B in the neutral species is not aromatic or could have some antiaromatic character. On the other hand, the results obtained for the oxidized form are somewhat striking. The previous MINDO/3 and HF/3-21G calculations[27] predict bent conformations for O_f^{2+},

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[57] A simple way to quantify the degree of pyramidal character is by defining the planarity defect at the N atom as $360° - \Sigma$(angles around the N atom). With this definition, the MP2 method predicts planarity defects of 16.5 and 27.4° for N(5) and N(10), respectively, whereas the DFT method gives planarity defects of 14.2 and 0.8°, respectively.

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TABLE 2. NICSs[a] (in ppm) at the Ring Critical Points, Anisotropy of the Magnetic Susceptibility[b] (χ_{anis}, in cgs-ppm), and Bird Indexes

	NICS(A)	NICS(B)	NICS(C)	χ_{anis}	I_A(A)	I_A(B)	I_A(C)	I_A(total)
$\mathrm{O_f}$	−10.1	−1.8	2.2	−118.7	88.9	69.7	65.0	223.6
$\mathrm{O_f}^{2+}$	10.3	1.4	3.4	−82.6	67.7	62.1	48.2	178.0
$\mathrm{R_f}$	−11.0	−0.4	−3.1	−76.9	95.1	36.6	54.5	186.2
$\mathrm{R_f}^{2+}$	−9.7	−8.8	0.1	−125.1	83.1	74.6	60.7	218.4
$\mathrm{R_c}$	−8.6	9.0	−3.9	−40.4	93.5	49.0	56.3	198.8

[a] Calculated at the GIAO-SCF/6-31+G(d)//MP2/6-31G(d,p) level for rings A−C. [b] Calculated at the IGAIM-SCF/6-31+G(d)//MP2/6-31G(d,p) level.

in contrast with the present results, which show planar C_s structures for both the MP2 and B3LYP methods. The relative energy for the constrained, folded structure $\mathrm{O_c}^{2+}$ is about 5 kcal/mol. Obviously, these results do not provide insight into the aromatic or nonaromatic nature of the neutral oxidized form.

The results obtained by the two magnetic criteria employed in this study, that is, NICSs[40] and the anisotropy of the magnetic susceptibility,[41,42] are collected in Table 2. Aromatic compounds have negative NICSs and quite large negative χ_{anis} values, whereas highly antiaromatic compounds have positive values of NICSs and χ_{anis}. The NICSs obtained for $\mathrm{O_f}$ show that ring A is aromatic (−10.1 ppm), as expected. However, the values for rings B and C are rather close to zero (−1.8 and 2.2 ppm, respectively), suggesting that these rings have no clear tendency for aromaticity or antiaromaticity. The results for $\mathrm{R_f}$ are quite similar: ring A is found to be aromatic (−11.0 ppm), and rings B and C do not present a clear tendency for aromaticity or antiaromaticity. The values of the anisotropy of the magnetic susceptibility predict that all the structures studied here are, as a whole, aromatic, with $\mathrm{R_f}^{2+}$ (−125.1 cgs-ppm) and $\mathrm{O_f}$ (−118.7 cgs-ppm) being the most aromatic ones. The significant decrease in χ_{anis} from the folded $\mathrm{R_f}$ structure (−76.9 cgs-ppm) to the constrained, planar $\mathrm{R_c}$ structure (−40.4 cgs-ppm) as well as the large positive NICS for ring B in the latter structure (9.0 ppm) suggest that the reduced form tends to adopt a bent conformation so as to relieve electronic repulsion in ring B, that is, avoiding to a certain degree an antiaromatic, planar structure for ring B. The results for the 2+ charged species are quite interesting. With the NICS method, ring A in $\mathrm{O_f}^{2+}$ is found to be antiaromatic (10.3 ppm), even though the conformation is planar. By contrast, the magnetic criteria predict $\mathrm{R_f}^{2+}$ as the most aromatic species; besides ring A, ring B is also found to be aromatic (NICS(B) = −8.8 ppm), a result consistent with the tendency toward planarity exhibited by this species. From the comparison between the results obtained for the neutral and 2+ charged species, it seems reasonable to conclude that the systems that are being studied here satisfy the $4n + 2$ rule to a certain extent. In addition, our results point out that, as a whole, the (neutral) oxidized form has more aromatic character than the (neutral) reduced form.

For the sake of comparison, we have also applied a geometry-based criterion (bond length equalization) for aromaticity, namely, the unified Bird index (I_A).[43] The higher the Bird index is, the more aromatic character the compound has. The values for rings A−C and for the global system are included in Table 2. The total values, I_A(total), for the full-optimized structures (neutral and charged species) follow the above trend: $\mathrm{O_f}$ is more aromatic than $\mathrm{R_f}$, and, when two electrons are removed, $\mathrm{R_f}^{2+}$ becomes more aromatic than $\mathrm{O_f}^{2+}$. Also, ring A is the most aromatic part of the isoalloxazine system, as expected. It may be instructive to show that the values for I_A(B) and I_A(total) increase in the neutral reduced form when planarity is imposed in the calculations ($\mathrm{R_c}$), suggesting an increase in aromaticity with planarity, which contrasts with the trend predicted by the magnetic criteria. However, as noted by Krygowski and Cyrański,[60] some paradoxical situations may appear when molecular geometry-based indexes are applied to nonequilibrium geometries; this could be an example of such paradoxical cases.

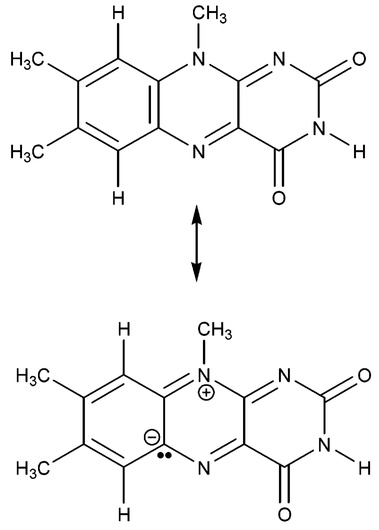

FIGURE 3. Best Lewis structures for the oxidized form ($\mathrm{O_f}$) obtained from NBO calculations using the MP2/6-31G(d,p) electron density.

We also performed natural bond orbital analyses[44−46] for the above structures using the MP2 and B3LYP electron densities. It is important to note that, due to resonance, all of the species studied here show an important role of valence non-Lewis orbitals (3.5−4.5%), so that their representations by Lewis structures have certain limitations. The NBO analysis for the MP2/6-31G(d,p) electron density of $\mathrm{O_f}$ affords two "best" Lewis structures with similar accuracy, which are displayed in Figure 3. One of the structures in the figure is the usual valence-bond representation for the oxidized form (Figure 3a). The other, unexpected structure (Figure 3b) stresses that the lone pair of N(10) is delocalized to a certain extent. The NBO analysis for B3LYP, however, shows only the former as the best Lewis structure. The Wiberg bond orders presented in Figure 4 corroborate that ring A is the only aromatic part of the molecule.

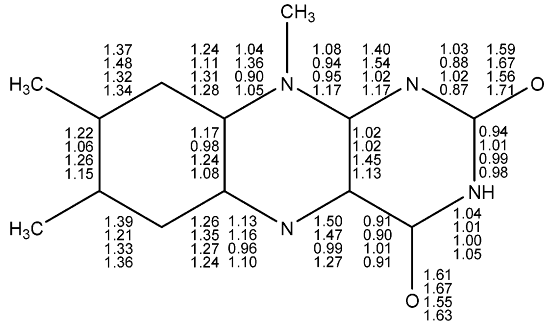

FIGURE 4. Wiberg bond orders for the species studied here obtained from NBO calculations using the MP2/6-31G(d,p) electron densities. From upper to lower values: O_f, O_f^{2+}, R_f, and R_f^{2+}.

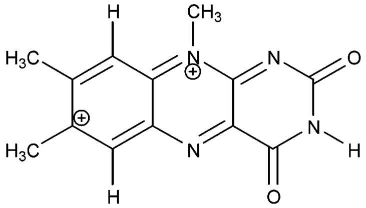

FIGURE 5. Best Lewis structure for O_f^{2+} obtained from NBO calculations using the MP2/6-31G(d,p) electron density.

The best Lewis structure for O_f^{2+}, obtained with the MP2 electron density, is depicted in Figure 5. The corresponding bond orders (see Figure 4) suggest a very low delocalization in this species. The loss of virtually one electron in ring A led to two nearly localized double-bonds, with Wiberg bond orders of 1.48 and 1.35. The N(5) and N(10) atoms, showing sp^2 hybridization, participate in two separate double bonds in ring B. The double bonds along the entire structure and the formal charge at C(7) are consistent with sp^2 hybridizations for the ring atoms and, consequently, with a planar geometry, even though antiaromaticity is clearly present in ring A.

For the reduced form, the best Lewis structure obtained from the MP2 electron density is the usual valence-bond representation, displayed in Figure 1. The Wiberg bond orders (see Figure 4) corresponding to ring A are all very similar to each other (values ranging from 1.24 to 1.33), in agreement with an aromatic character. For ring B, the results predict a double bond, the C(4a)−C(10a) bond (bond order of 1.45), with the remaining bonds showing single-bond character [except for C(5a)−C(9a), which presents a bond order of 1.24]. The corresponding bond orders obtained from the B3LYP electron density are slightly higher, indicating a slight tendency toward aromaticity with respect to the MP2 structure. According to the s−p contributions obtained by the NBO analysis for the MP2 density, N(10) presents significant sp^3 character, as expected from the planarity defect calculated for this atom (27.4°).[57] The N(5) atom shows a lower sp^3 character, as one could also anticipate from the geometries around these atoms. By contrast, the NBO analysis for the B3LYP density predicts sp^2 character for N(10), which is also expected since the geometry around N(10) is essentially planar at this level of calculation.

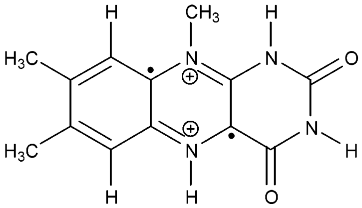

FIGURE 6. Best Lewis structure for R_f^{2+} obtained from NBO calculations using the MP2/6-31G(d,p) electron density.

Finally, Figure 6 depicts the best Lewis structure for R_f^{2+}. The most remarkable difference with respect to the neutral species is the loss of two electrons in ring B. As a result, the delocalization around ring B increases substantially, which is exhibited by the fact that the differences between the bond orders are rather small. As compensation, the delocalization around rings A and C decreases, but only slightly, so that, as a whole, there is an increase in aromaticity.

4. Conclusions

The MP2 and B3LYP methods in conjunction with the standard 6-31G(d,p) basis set were used to investigate the molecular structure of flavins, in its oxidized and reduced forms, using lumiflavin as a model compound. The study was aimed at elucidating the possible aromatic properties of these systems. To this end, we applied to the above systems several criteria for aromaticity: NICSs,[40] the anisotropy of the magnetic susceptibility,[41,42] the unified Bird Index,[43] and the natural bond orbital approach.[44−46] To provide further insight, calculations were also performed on the 2+ charged species.

The B3LYP and MP2 calculations predicted planar structures for the oxidized form and bent conformations for the reduced form, with fold angles of ca. 25 and 30°, respectively. The MP2 calculations showed a pyramidal N(10) atom, whereas the DFT computations afforded an essentially planar geometry around N(10). The barrier for ring inversion was estimated to be 6.9 and 2.3 kcal/mol at the MP2 and B3LYP levels, respectively.

The aromaticity indexes employed in this study indicate that, as a whole, the oxidized form is more aromatic than the reduced form and that ring A is clearly the most aromatic part of the flavins, as expected. The calculations predicted that the ring folding in the reduced form appears mainly as a result of minimizing electronic repulsions in ring B: a planar structure would lead this ring to experience some antiaromatic character (this trend is stronger at the MP2 level than at the B3LYP level).

Finally, the calculations showed that removing two electrons from the neutral species significantly affects the aromatic properties of lumiflavin, suggesting that this system fulfils the Hückel rule to a certain degree.

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Supporting Information Available: Geometries and total energies for the species studied. This material is available free of charge via the Internet at http://pubs.acs.org.

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