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Ab initio molecular orbital studies of closed shell flavins

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Abstract. Ab initio calculations with a $(7s\ 3p)$ basis set are performed on uracil, lumazine, alloxazine and various isoalloxazines. The results as total energies and charge distributions are discussed in relation to the biochemical behaviour of the flavins. The calculations correctly predict equilibrium situations in the alloxazine-isoalloxazine system and explain the high affinity for nucleophilic addition at N_5 in the flavins. The reduction of flavins and their reoxidation by oxygen are discussed.

Key words: Ab initio, molecular orbital, electronic structure, flavin, isoalloxazine

Introduction

Flavins (substituted isoalloxazines, Fig. 1) play an important role in almost all living organisms. They act as cofactor for flavoproteins in many metabolic redox reactions and participate as such in the respiratory chain (Lehninger 1976). Model studies have revealed that isoalloxazines have a high affinity for nucleophilic addition at nitrogen 5 (Müller et al. 1969; Müller and Massey 1971; Müller 1972). At this position the molecule is highly electrophilic and reacts for instance with hydride ions. This property of the flavins is unique amongst N-heterocyclic compounds and is essential for the biochemical function of the flavoproteins. Examples of such biochemical reactions are the dehydrogenase reactions in the Krebs cycle where hydride ions are transferred to the flavin moiety of the enzyme, thereby reducing the isoalloxazine system (Lehninger 1976).

To investigate the electronic structure of closed shell flavins we performed ab initio SCF-MO (self consistent field molecular orbital) studies for a number of isoalloxazines and some related smaller compounds such as uracil and lumazine. The results of these calculations in relation to photo-electron spectroscopy have been published separately (Palmer and Platenkamp 1979; Palmer et al. 1980, 1982). Here we focus our attention on total energies and charge densities. Interpretation of these quantum mechanical results provides some insight into flavin biochemical reactivity. The present ab initio SCF-MO study allows a more detailed and accurate description of the unusual electrophilic affinity of N_5 than previous semi-empirical calculations in which only π-electrons (Pullman and Pullman 1959; Karreman 1961; Grabe 1972, 1974; Sun and Song 1973, 1974) or valence electrons (Song 1968, 1971; Eweg et al. 1980) are treated.

The molecules described in this investigation are alloxazine, isoalloxazine, its 10-methyl derivative, the reduced 1,5-dihydro-isoalloxazine and several isoalloxazine ions of relevance to flavin biochem-

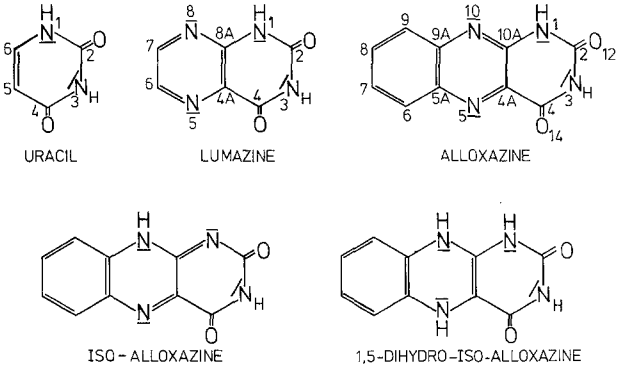

Fig. 1. Structure formulas and numbering systems of uracil, lumazine and the (iso)-alloxazines. Note that if both N_1 and N_{10} carry a substituent the difference between isoalloxazines and alloxazines is lost

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istry. Related smaller compounds such as uracil and lumazine are also treated. The structural formulas of these molecules and their numbering systems are given in Fig. 1.

Methods

Molecular structures

Uracil and lumazine were studied at their crystal structure geometry (Stewart and Jensen 1967; Norrestam et al. 1972). The structure of alloxazine was obtained by fusion of a benzene ring (CC = 1.398 Å) onto lumazine. Isoalloxazine and its 10-methyl derivative were based upon the crystal structures of lumiflavin (Norrestam and Stensland 1972; Leijonmarck 1977) with minor deviations from planarity removed (Fritchie 1975). The reduced 1,5-dihydroisoalloxazine has been studied at various geometric arrangements, both planar and non-planar; from crystal structure studies it is known that this molecule is non-planar in the solid state with a "butterfly" shape (see Fig. 2). The folding angle Φ and the positions of the 5H and 10H atoms (axial or equatorial) were treated as variables.

Computations

The major differences between the present work and previous calculations are that all electrons are included and that all the integrals have been explicitly evaluated without the introduction of semi-empirical parameters or experimental data. The atomic orbitals for the elements C, N and O were represented by a minimal $(7s\,3p)$ basis, consisting of 5, 2 and 3 (×3) Gaussian Type Orbitals (GTO's) for $1s$, $2s$ and $2p$ orbitals respectively, while $1s_H$ was represented by 3 GTO's. The exponents and contraction coefficients have in all cases been optimized on small molecules (Palmer et al. 1974, 1975). Where comparison has been made the present basis yields energies significantly lower than the STO-3G basis and close to the Pople 4-31G double zeta basis (Ditchfield et al. 1971).

As a result of its smaller size, it was practical to carry out another larger calculation on uracil in which the $(9s\,5p)$ double zeta basis was used (Dunning 1970). This showed that, although the energy difference between the minimal and double zeta basis set is large (see Table 1), various molecular properties such as dipole moment, orbital energies and ordering and atomic populations prove to be practically the same for both calculations (Palmer et al. 1980).

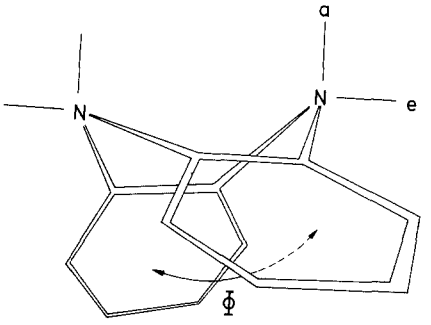


Fig. 2. Folding angle Φ and axial and equatorial substituent positions in reduced flavins

All computations were carried out at the computer centre of Edinburgh University on an IBM 370/168 computer using the ATMOL-3 system.

Results and discussion

Total energies

The molecular total energies have been collected in Table 1. The isoalloxazine species occurring in this table that possibly are involved in flavin biochemistry have been gathered in Fig. 3 to show the relationships between the various forms. The Roman numeral I refers to oxidized, II to one-electron reduced and III to two-electron reduced isoalloxazine systems, whereas the symbols A, B and C refer to protonation.

The presence of a 10-substituent is essential for the redox activity of the flavins (Lehninger 1976) and it is of interest to have an estimate of the energy difference between the tautomers isoalloxazine and alloxazine. From Table 1 we derive a value of 235 kJ mol^{-1} with alloxazine being the more stable. Since the geometry of alloxazine was based upon lumazine, while for isoalloxazine the experimental geometry of 7,8,10-*tri*-methyl-isoalloxazine was used, this value represents a lower limit and strongly indicates that 10-unsubstituted isoalloxazines do not exist. The large difference in total energy can be ascribed to the presence of a quinoxaline residue in alloxazine which accounts for a larger resonance energy with respect to isoalloxazine.

The calculations predict correctly that monoprotonation of isoalloxazine leads to a 1H-protonated form (IA) rather than a 5H-protonated form (IC), in agreement with experimental evidence (Dudley et al. 1964). The calculated energy difference is 170 kJ mol^{-1}. Although the (possible) adoption of a butterfly shape could lower the total energy of the 5H-cation, the gain is unlikely to exceed 120 kJ mol^{-1}

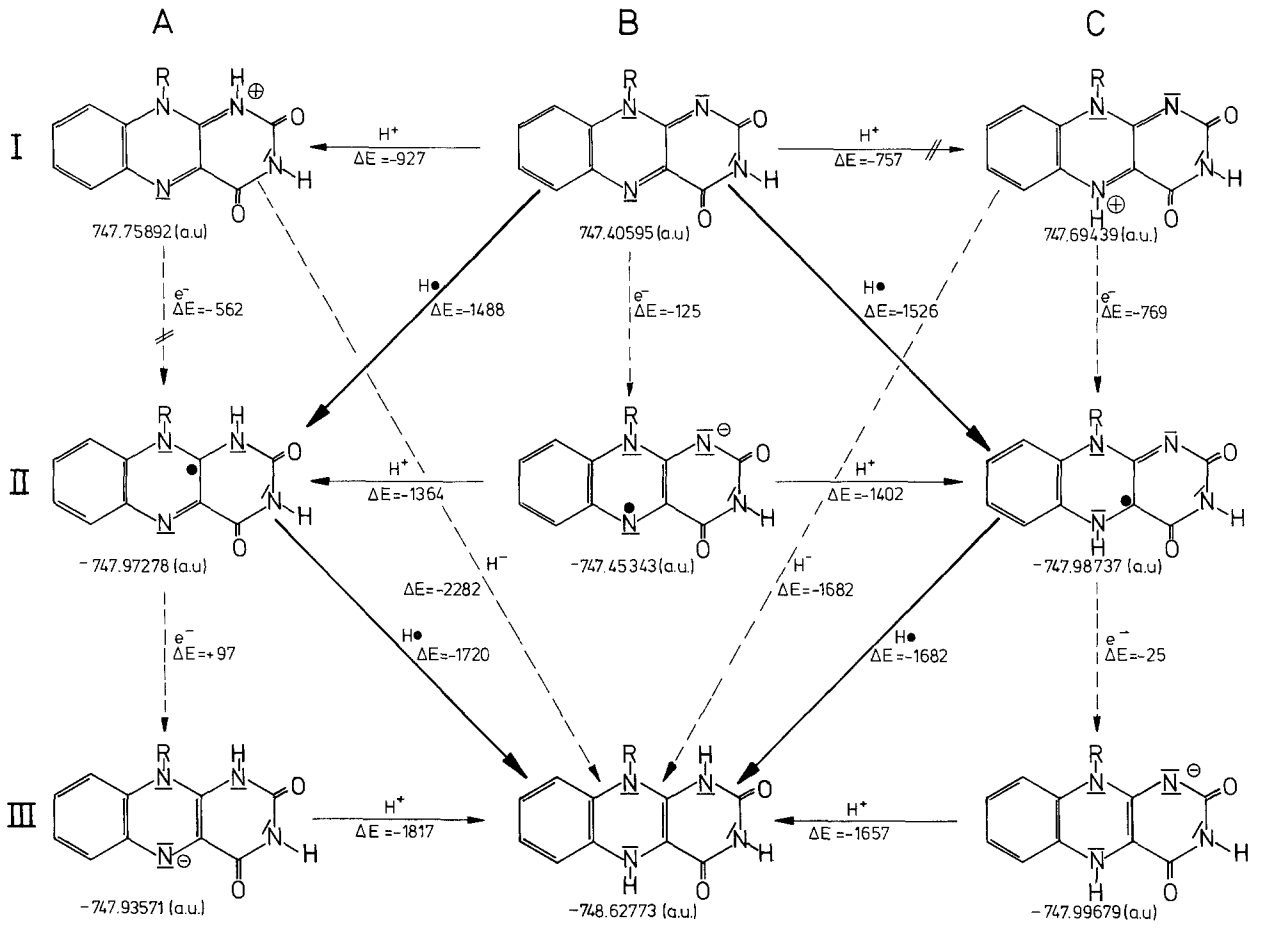


Fig. 3. Total energies (in atomic units) of oxidized isoalloxazines (IA, B, C), one electron reduced isoalloxazines (IIA, B, C) and two electron reduced isoalloxazines (IIIA, B, C). Various pathways from oxidized isoalloxazine (IB) to the fully reduced 1,5-dihydroisoalloxazine (IIIB) are indicated. The energy change in the isoalloxazine system is given in kJ mol^{-1} for each step

(see reduced compound (IIIB)). Hence, it is clear that the planar 1H-cation (IA) is preferred in an equilibrium situation.

The molecular total energies also provide insight into the equilibrium reactions between oxidized and reduced flavins. For instance, the reaction:

$$Fl_{ox} + FlH_2 \rightleftarrows 2FlH^{\cdot}$$

between oxidized flavin (Fl_{ox}, cf. IB) and reduced flavin (FlH_2, cf. IIIB) is theoretically predicted to lead to disproportionation, the energy difference favouring the left hand side being 155 kJ mol^{-1}. Experimentally the neutral radical indeed disproportionates according to the above reaction in the pH range 4–10 (Müller et al. 1970).

On the other hand, the reaction between 5H-protonated derivatives (cf. IC and IIIC) theoretically favours the neutral radicals (IIC) by a very large amount (676 kJ mol^{-1}). Experiments using blocked 5-methyl derivatives show that the equilibrium of the reaction is shifted towards the radical form (Bruice and Yano 1975), in agreement with our calculations.

The geometry of 1,5-dihydro-isoalloxazine

It has been known for a long time that the fully reduced flavins (cf. IIIB) adopt a non-planar conformation. A non-planar geometry was first suggested by Dudley et al. from *light* absorption data (Dudley et al. 1964). This idea was confirmed via X-ray studies by Kierkegaard and co-workers (Norrestam et al. 1969; Kierkegaard et al. 1971), who established a folding angle $\Phi = 148°$ in the solid state. In later X-ray studies some spread of the angle Φ with the nature of the substituents was observed (Norrestam and Stensland 1972; Leijonmarck 1977).

We performed calculations with interplanar angles Φ of 148°, 164° and 180°, whereas the question of the stereochemistry of the NH-bonds was treated in detail. The discussion of the energy surfaces will be published separately, here we shall confine ourselves to the main points. The present calculations as well as those on the radicals (Platenkamp et al. 1980) show that in all instances reduction of the planar isoalloxazine (cf. I) leads to a bent conformation with $\Phi \approx 164°$ as illustrated in Fig. 4.

Table 1. Calculated total energies. Roman numerals refer to Fig.3

Molecular total energies (a. u. [a]*)*

Molecule			E_T
Isoalloxazine		IB	− 747.40595
10 Me-isoalloxazine			− 786.30000
1,5-Dihydro-isoalloxazine		IIIB	
(*a*) planar N_5/N_{10} interplanar angle Φ	148°		− 748.59844
	164°		− 748.62732
	180°		− 748.57929
(*b*) $5H_{eq}\,10H_{ax}$	164°		− 748.57405
(*c*) $10H_{eq}\,5H_{ax}$	164°		− 748.62773
Alloxazine			− 747.49551
1 H-isoalloxazine cation		IA	− 747.75892
5 H-isoalloxazine cation		IC	− 747.69439
1 H-neutral radical	180	IIA	− 747.97278
5 H-neutral radical	164	IIC	− 747.98737
anion radical	164	IIB	− 747.45343
1 H-isoalloxazine anion		IIIA	− 747.93571
5 H-isoalloxazine anion		IIIC	− 747.99679
Isoalloxazine dianion interplanar angle Φ	148		− 747.23351
	164		− 747.26494
	180		− 747.23441
Uracil			
minimal basis			− 411.07831
double zeta basis			− 412.23909
Lumazine			− 595.16374

[a] a. u. = 27.2 eV = 2,626 kJ mol^{-1}

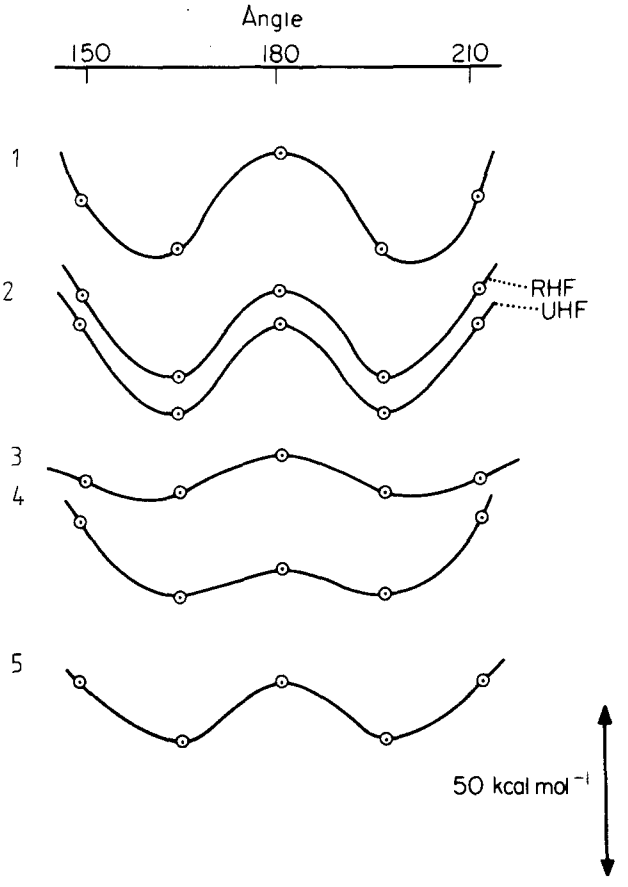


Fig. 4. Ring inversion barriers calculated for isoalloxazines. See also Platenkamp et al. (1980) for the radicals. 1) 1,5-dihydro-isoalloxazine (IIIB); 2) isoalloxazine cation radical; 3) isoalloxazine anion radical (IIB); 4) neutral radical (IIC); 5) isoalloxazine dianion

Hence, not only the fully reduced 1,5-dihydro-isoalloxazine and isoalloxazine dianion but also the anion radical (IIB), neutral radical (IIC) and cation radical are non-planar with the same approximate angle of 164°. The calculated vapour phase geometry of the reduced 1,5-dihydro-isoalloxazine differs slightly from the solid state geometry. Such a difference between the solid state and vapour or liquid phase geometries is not uncommon for this type of compound. A similar change in geometry for instance has been found in molecules like dibenzdioxine and other phenoxachalcogenins (Colonna et al. 1978).

It is interesting to note that an earlier extended Hückel MO calculation predicted a value of 148° (Norrestam et al. 1969). A study of the interplanar angle Φ by the 'ab initio' PRDDO (partial retention of diatomic differential overlap) method resulted in an angle $\Phi = 165°$, in agreement with our results (Dixon et al. 1979). All these results contrast with those of Eweg et al. (1980) who proposed a planar conformation of reduced flavins, based upon a CNDO (complete neglect of differential overlap) study.

When separating the total energy into its components, electronic energy and nuclear repulsion, we may see the origin of non-planarity of the dihydroflavin. For 1,5-dihydro-isoalloxazine the figures are as follows (a. u.): nuclear repulsion 1,062.2515 (180°), 1,058.0092 (164°), 1,064.3411 (148°); electronic energy −1,810.8308 (180°), −1,806.6366 (164°), −1,811.9396 (148°). Using parabolic extrapolation we find that the *nuclear* repulsion energy is minimized at the total energy minimum, whereas the *electronic* energy is at its maximum at this position (165°). The same situation is encountered in the isoalloxazine radicals (Platenkamp et al. 1980) and in the isoalloxazine-dianion. This shows that the potential anti-aromaticity (7 or 8 π-electrons inside the pyrazine ring) (Tauscher et al. 1973) is not responsible for the non-planarity of these molecules.

With respect to the substituent positions, it is interesting to note that the 5,10-di-equatorial compound (*e*, *e*) is favoured over the other conformers

by approximately 141 (5*e*, 10*a*), 157 (5*a*, 10*e*) and 267 (5*a*, 10*a*) kJ mol^{-1}. However, we have to note that this only holds for protons, bulkier substituents will probably be axial because of steric hindrance by the 4CO group and non H-substituents on C_9 and N_1. Hence, the reduced flavins occurring in nature should adopt a (5*e*, 10*a*) conformation.

Charge densities

The relevant charge densities may be found in Table 2, where π- and total populations are given.

Electron distribution in uracil, lumazine and alloxazine

In general the atomic populations change by comparatively small amounts on going from uracil to lumazine and alloxazine. This is true in particular for the π-density. In the σ-system there are some small changes. *On going from uracil to lumazine the populations on* C_5 *and* C_6 *are lowered by about 0.25 e.* The change from lumazine to alloxazine is similar, although the changes on C_6 and C_7 of lumazine are smaller (0.12 *e*) because of the lower polarity of the benzene ring. Each of the NH groups in these molecules is like that in pyrrole, being a σ-acceptor and π-donor.

A direct comparison of the dipole moments for the series uracil, lumazine, alloxazine and isoalloxazine is made in Fig. 5. The principal difference is in the magnitude (shown to scale), the dipole moment directions are rather similar. Previously it has been shown that the basis set we employed gives very realistic results for the charge densities and moments of heterocycles (Palmer et al. 1974, 1975) and that the value calculated for the dipole moment arises from a delicate balance of the internal bond dipoles (Palmer et al. 1974).

There is general agreement between our results and semi-empirical studies on the dipole moment directions (Grabe 1972, 1974; Song 1968, 1971). Comparison with all-electron calculations can only be made for uracil and isoalloxazine. A FSGO (floating spherical Gaussian orbital) calculation resulted in a dipole moment of 2.27 D for uracil (O'Donnell et al. 1978), to be compared with the experimental value of 4.16 D (Kulakowska et al. 1974) and our value of 4.5 D. In the case of isoalloxazine the experimental values is not known. Almost all semi-empirical calculations predict a very large dipole moment between 5 and 8 D in agreement with our value of 7.8 D, but sharply in contrast with the 'ab initio' PRDDO result of 2 D (Dixon et al. 1979).

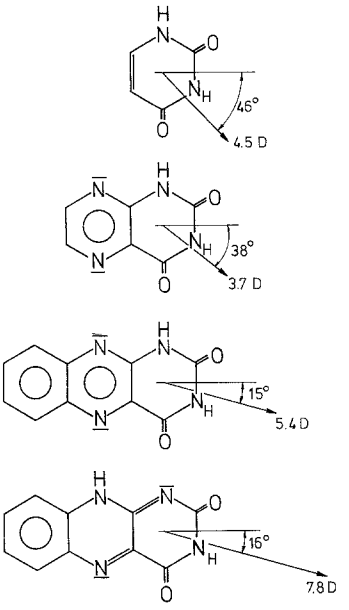


Fig. 5. Calculated dipole moments (in debyes) of uracil, lumazine, alloxazine and isoalloxazine

Electron distribution in the isoalloxazine system

With the obvious exceptions of N_1 and N_{10} the atomic populations of isoalloxazine and alloxazine are very similar. One remarkable feature is the difference between N_1 and N_5 in isoalloxazine. Both nitrogens are nominally pyridine type, but there is a difference of 0.15 *e* in the total populations, N_1 being the more electron rich. The π-electron difference is even larger, with N_1 having 0.36 π-electrons more. In this respect N_5 is clearly unusual; these values are not an artefact of isoalloxazine, since the same phenomenon is observed in 10 *Me*-isoalloxazine, although the 10-methyl group is a significantly weaker donor (0.24 *e*) to the σ-system than the 10-hydrogen (0.34 *e*). Thus, although N_5 is negative overall, it is less negative than usual in *N*-heterocyclic compounds and it bears a π-electron deficiency. It is woth noting that this theoretical finding receives support from the interpretation of the photo-electron spectrum of isoalloxazine (Palmer et al. 1980, 1982). The remarkable affinity of N_5 in isoalloxazine for nucleophiles and in particular H^- is obviously due to a large extent to this particular distribution. We have to note of course, that the corresponding nitrogen-5 atoms in alloxazine and lumazine have a similar charge distribution. However, because of their more aromatic character these compounds are unable to react with nucleophiles at the N_5 position.

Reduction to 1,5-dihydro-isoalloxazine causes only minor changes in the charge distribution, except on

Table 2. Total and π-populations for uracil, lumazine, alloxazine and isoalloxazine (IB)

(*a*)	*Uracil*					
	O(2)	O(4)	N(1)	N(3)		
π	1.408	1.352	1.737	1.729		
Total	8.515	8.490	7.466	7.461		
	C(2)	C(4)	C(5)	C(6)		
π	0.863	0.861	1.137	0.912		
Total	5.380	5.539	6.232	5.970		
	H(1)	H(3)	H(5)	H(6)		
Total	0.672	0.666	0.808	0.800		
(*b*)	*Lumazine*					
	O(2)	O(4)	N(1)	N(3)	N(5)	N(8)
π	1.412	1.311	1.741	1.728	0.940	1.067
Total	8.514	8.462	7.449	7.444	7.196	7.261
	C(2)	C(4)	C(4a)	C(6)	C(7)	C(8a)
π	0.865	0.868	1.111	1.047	0.958	0.952
Total	5.401	5.522	5.950	6.066	6.035	5.746
	H(1)	H(3)	H(6)	H(7)		
Total	0.661	0.660	0.813	0.818		
(*c*)	*Alloxazine*					
	O(12)	O(14)	N(1)	N(3)	N(5)	N(10)
π	1.415	1.312	1.745	1.731	0.939	1.089
Total	8.519	8.466	7.451	7.446	7.194	7.272
	C(2)	C(4)	C(4a)	C(5a)	C(6)	C(7)
π	0.864	0.866	1.113	1.064	0.961	0.993
Total	5.403	5.524	5.954	5.944	6.128	6.163
	C(8)	C(9)	C(9a)	C(10a)		
π	0.949	1.000	0.984	0.972		
Total	6.148	6.143	5.914	5.760		
	H(1)	H(3)	H(6)	H(7)	H(8)	H(9)
Total	0.664	0.663	0.802	0.818	0.814	0.808
(*d*)	*Isoalloxazine (IB)*					
	O(12)	O(14)	N(1)	N(3)	N(5)	N(10)
π	1.332	1.325	1.258	1.731	0.898	1.680
Total	8.490	8.473	7.347	7.454	7.193	7.404
	C(2)	C(4)	C(4a)	C(5a)	C(6)	C(7)
π	0.894	0.870	1.090	1.118	0.934	1.038
Total	5.448	5.531	5.939	5.942	6.123	6.182
	C(8)	C(9)	C(9a)	C(10a)		
π	0.926	1.063	0.942	0.900		
Total	6.123	6.178	5.857	5.727		
	H(3)	H(6)	H(7)	H(8)	H(9)	H(10)
Total	0.670	0.809	0.826	0.819	0.818	0.666
(*e*)	*10 Methyl-isoalloxazine (partial results)*					
	O(12)	O(14)	N(1)	N(3)	N(5)	N(10)
π	1.339	1.327	1.276	1.731	0.901	1.641
Total	8.496	8.477	7.357	7.454	7.175	7.280
	C(Me)	H(Me)				
Total	6.353	0.801				

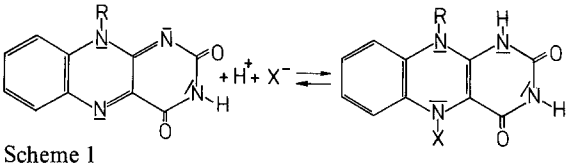
Table 2 (continued)

(*f*)	*Reduced iso-alloxazine (III B)*					
	O(12)	O(14)	N(1)	N(3)	N(5)	N(10)
π	1.436	1.406	1.695	1.693	1.716	1.693
Total	8.535	8.512	7.439	7.443	7.447	7.441
	C(2)	C(4)	C(4a)	C(5a)	C(6)	C(7)
π	0.866	0.876	1.188	0.981	1.044	1.006
Total	5.405	5.549	5.977	5.875	6.183	6.166
	C(8)	C(9)	C(9a)	C(10a)		
π	1.032	1.013	1.038	0.931		
Total	6.175	6.162	5.902	5.715		
	H(1)	H(3)	H(5)	H(6)	H(7)	H(8)
Total	0.665	0.675	0.702	0.833	0.835	0.833
	H(9)	H(10)				
Total	0.831	0.700				

N_1 and N_5. Both nitrogens change from pyridine type to pyrrole type, becoming σ-acceptor and π-donor. The principal beneficiary of π-donation is C_{4A} ($-0.19e$), a fact that could be of relevance to the natural reoxidation by oxygen. The charge distribution itself is hardly affected by changes in the folding angle Φ, just as in the flavin radicals (Platenkamp et al. 1980).

Reactivity

There is a vast body of experimental knowledge of flavin chemistry, and excellent reviews exist (Bruice 1976, 1980; Walsh 1978; Ghisla 1982; Hemmerich et al. 1982; Müller 1983). In this section we will focus our attention on the reduction of oxidized flavin, according to the reaction:



Scheme 1

in which X^- is a nucleophile. In this reaction two electrons are accepted by the flavin π-electron system. Apart from its biochemical importance, this reaction is interesting from a theoretical point of view. A nucleophile (X^-) here reacts with N_5, representing a unique property of the flavins: generally nitrogen atoms behave like a Lewis base, but N_5 apparently acts as a Lewis acid. The detailed mechanism of the reduction of isoalloxazines is not yet established, even in model reactions, such as reduction by borohydride (hydride transfer) (Müller et al. 1969), sulfite (Müller and Massey 1971) or tri-

phenylphosphine (Müller 1972). In enzymatic reactions the nucleophiles can be carbanions (Porter et al. 1973; Walsh 1978), or a hydride-ion in the well known NADH-dehydrogenase reaction (Lehninger 1976; Walsh 1978; Ghisla 1982).

The major unresolved question is how electrons are transferred from the substrate X^- to the isoalloxazine nucleus. Arguments exist in favour of covalent adducts (Porter et al. 1973), charge-transfer complexes have been proposed (Massey and Ghisla 1974), and the idea has been advanced that the two-electron reduction occurs one electron at a time, to produce the flavin semiquinone and substrate radical as intermediate species, before the passage of the second electron and spin pairing (Bruice 1976; Williams and Bruice 1976).

Charge-density and frontier orbital considerations

The first question, the electron acceptor capacity at N_5, can be explained with the present theoretical results. In the previous section it was shown that N_5, although overall negative, is electron deficient in the π-system. This is verified experimentally from the photo-electron spectrum: the lone pair electrons at N_5 are more tightly bound than the N_1 lone pair electrons, as shown by their respective ionization levels (10.4 eV for N_1 and 12.6 eV for N_5) (Palmer et al. 1980, 1982). This electron deficiency of N_5 partly explains its electrophilic character.

In Fig. 6 we have depicted the coefficients of the HOMO (highest occupied MO) and LUMO (lowest unoccupied MO) of isoalloxazine. These orbitals contain diaza-buta-1,3-diene character. The $-N=C-C=N-$portion of the HOMO as compared to the diazabutadiene HOMO has a markedly larger density on N_1 than on N_5. This is consistent with ready protonation at N_1. The LUMO of isoalloxazine has a large amount of diazabutadiene-LUMO character but now has its density shifted towards N_5.

In a simple MO-description the reducing electrons have to be accommodated in the LUMO of the oxidized flavin. Inspection of the LUMO shows that the Frontier Orbital density for nucleophilic attack is by far the highest at N_5 (30%). This makes N_5 the preferred site for reduction (Flemming 1975). It implies that the reaction is orbital controlled, initiating with π-coordination at N_5 followed by rotation into the molecular plane, as suggested previously (Sun and Song 1973; Palmer and Platenkamp 1979). It is interesting to note that MO theories at different levels of sophistication, ranging from simple Hückel π-electron calculations to the present ab initio calculations, all predict the high frontier orbital density at N_5 (Pullman and Pullman 1959; Grabe 1972, 1974; Sun and Song 1973). It appears that the character of the LUMO is mainly dictated by the topology of the π-system, independent of the constituent atoms (R. J. Platenkamp unpublished results), and this explains why 5-deazaflavin can perform similar reactions (Sun and Song 1973). To get more solid evidence for the Frontier Orbital argument we performed ab initio calculations on the isoalloxazine mono- and dianions. If the argument applies the added electrons need to have a large density on N_5. This is indeed the result of the calculations: in the anion radical (IIB) at least 50% of the unpaired electron density is localized on N_5 (Platenkamp et al. 1980); in the isoalloxazine-dianion the HOMO is very similar to the parent LUMO and accounts for a density of 0.58 e at N_5:

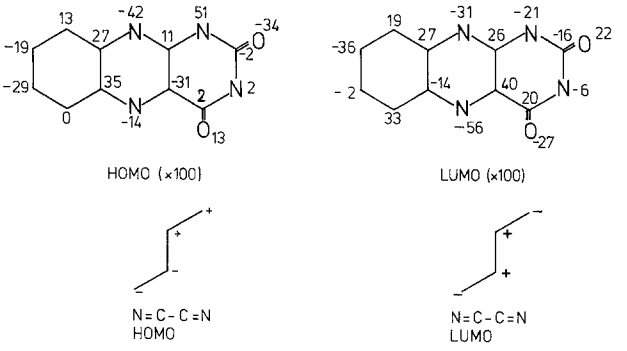
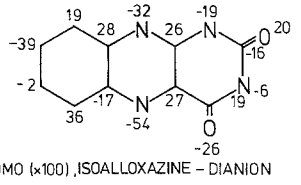


Fig. 6. HOMO and LUMO of isoalloxazine (IB). A qualitative comparison with the diazabutadiene HOMO and LUMO is made

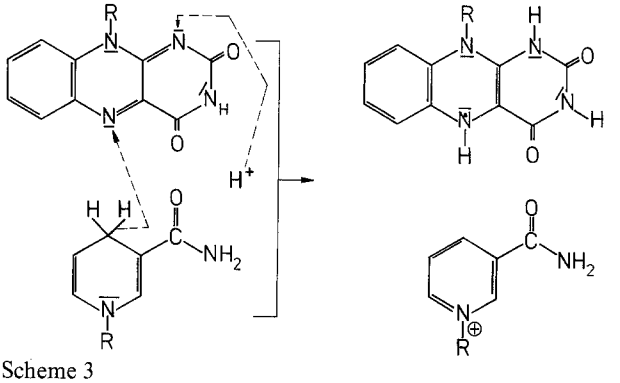


Scheme 2

Thus, the present calculations establish clearly that N_5 acts as the main 'electron sink' in isoalloxazine. The unique electrophilic character of N_5 stems from a low atomic population in the filled MO's (charge criterion) and an extremely high population in the lower empty π-MO's (orbital criterion). These conclusions lead to a mechanism involving protonation at N_1 (which increases the overall reactivity to nucleophiles) and subsequent 'orbital controlled' nucleophilic addition to N_5. This mechanism holds for the model reactions (phosphine, sulfite) and is thought to be true also for enzymatic reactions involving carbanions, such as catalysed by D-amino acid oxidase (Porter et al. 1973).

The NADH-dehydrogenase reaction

The above argument might also explain the mechanism of the dehydrogenase reaction, in which a hydride is transferred from the pyridine-nucleotide NADH to the flavin nucleus:



Scheme 3

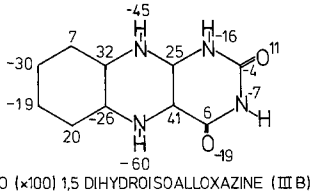
To provide some background for the discussion we have given, in Fig. 3 a general scheme in which various conceivable pathways are indicated along which reduction might proceed. All states are given with their calculated total energies in atomic units, whereas energy differences are given in kJ mol^{-1}. Out of this scheme the species IC, IIA and IIIA are not likely to be formed because of the existence of their much more stable isomers IA, IIC and IIIC. Therefore, we assume for the present that they do not occur as reaction intermediates. The only flavin semiquinone known to have a biological function is the neutral radical IIC. However, no radicals have been detected, neither during the NADH-dehydrogenase reaction, nor in model studies. Hence, for the present we don't take radicals into account as intermediates.

With these premisses, the reduction itself has to be a two-electron transfer. Our calculations then suggest the following mechanism: protonation at N_1 ($pK_a = 10$) followed by direct hydride transfer to N_5 with initial binding to the isoalloxazine π-system and subsequent rotation into the molecular plane. The electrons are thus transferred according to the covalent adduct mechanism (Porter et al. 1973; Walsh 1978).

This mechanism is consistent with the observed stereo specificity and results of deuterium exchange reaction (You et al. 1977). A point raised against direct hydride transfer from the pyridine nucleotide is the amount of energy needed to release H^- from NADH. This requires at least the ionization potential (≈ 8 eV). The gain in aromatic energy partially compensates this. Moreover it is known that in the metal hydride reduction of aryl ketones (Lansburg and Peterson 1963; Brown 1970) hydride ions are delivered from a 'NADH' type of reagent (lithium tetrakis-(*N*-dihydropyridyl)-aluminate), which provides indirect support for the above mechanism.

Oxidation of reduced flavin by triplet oxyen

Frontier Orbital considerations suggest that N_5, N_{10}, C_{4A}, C_{9A}, C_{5A} and C_{10A} in that order are the preferred sites for electrophilic attack. This is seen from the HOMO which has a high density at these centres:



HOMO (×100) 1,5 DIHYDROISOALLOXAZINE (IIIB)

Scheme 4

A high reactivity at these positions is indeed borne out by experiments in which C_{4A}, C_{9A} and C_{10A} adducts have been observed.

The distribution in the HOMO and the HOMO energy depend only slightly on the folding angle Φ. So, in this case the enzymatic activity is not strongly conformation dependent. Thus, although the apoenzyme and substrate binding may influence the conformation of the $FADH_2$-moiety, this will not influence the site of electrophilic attack, although it may change overall reactivity.

The natural reoxidation of reduced flavin by oxygen is not initiated by normal electrophilic attack because of the triplet ground state of oxygen. Kemal et al. (1977) have proposed a mechanism for this reaction that now seems generally accepted. It is gratifying to see that the present ab initio calculations support this mechanism depicted in Fig. 7.

First an anion is formed by deprotonation at N_1, in agreement with our calculations, which predict the N_1-anion (cf. IIIC) to be 162 kJ mol^{-1} more stable than the N_5-anion (cf. IIIA). The next step is the reaction with triplet oxygen ($^3\Sigma_g$) to form a triplet adduct. According to ESR experiments and our calculations, the isoalloxazine radicals do not carry any significant spin density on N_1, C_{9A} and C_{10A}, whereas C_{4A} carries a large spin density. Hence, in the Frontier Orbital picture (Flemming 1975) the triplet oxygen adduct should be formed at C_{4A} (orbital controlled via π-coordination). This is in agreement with the crucial step in the proposed mechanism. The reaction is terminated by spin pairing and deprotonation at N_5, breaking of the C_{4A}-oxygen bond and formation of H_2O_2.

Experimental evidence for a reduced flavin-peroxide adduct at C_{4A} has also been obtained for the

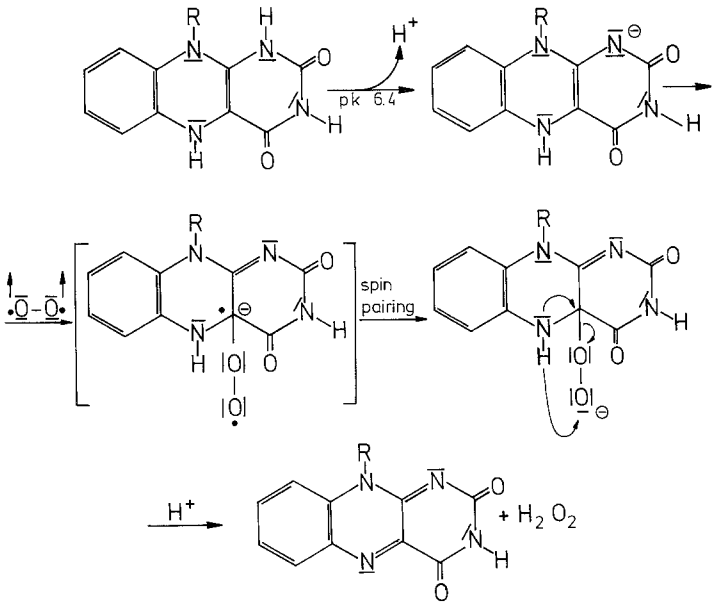


Fig. 7. Reoxidation of 1,5-dihydro-isoalloxazine (IIIB) to isoalloxazine (IB) by triplet oxygen according to Kemal et al. (1977)

reaction of superoxide ions with isoalloxazine radicals (Anderson 1982). This system is isoelectronic with the oxygen-reduced flavin system.

Concluding remarks

The present ab initio calculations give a reasonable description of the electronic structure of the flavins and have been a great help in the elucidation of photo-electron spectra of these compounds (Palmer et al. 1980, 1982). In this investigation it is shown that with the use of a well balanced minimal basis set $(7s\,3p)$ one may also account in a satisfactory manner for the chemical behaviour of these molecules. Equilibrium situations such as the alloxazine-isoalloxazine system are correctly predicted. The calculations provide deeper insight into the chemical reactivity of the flavins compared with semi-empirical calculations, and may be a guide in determining reaction mechanisms. It is tentatively concluded that reduction of oxidized isoalloxazine proceeds via covalent adducts (simultaneous transfer of two electrons), whereas the reoxidation by $^3\Sigma_g$ oxygen proceeds one electron at a time, via a triplet intermediate.

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