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Semi-empirical Self-consistent Field Calculations of Heteroaromatic Compounds of Biological Interest. II.[1] The π-Electronic Structure of Riboflavin

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The Pariser-Parr-Pople SCF calculation has been carried out with the aid of the variable β-core method for the investigation of the π-electronic structure of riboflavin. Comparison between the experimental and theoretical results on the transition energy and intensity, and the direction of transition moment of π-π^* bands in the electronic spectra is presented, together with the π-electron densities and free valences of the lowest singlet and triplet excited states which have been calculated with the aid of configuration interaction method. It has been found that the π-electron distribution of the photo-excited state is in parallel with the experimental facts suggesting a photo-induced change of basicity and a direct hydrogen-transfer in anaerobic photobleaching reaction.

Introduction

Investigations of the π-electronic structures of riboflavin and its related compounds are regarded to be of much importance in giving theoretical background to the complicated mechanism for their essential role in oxido-reductive processes in many models and biological systems, and also for the quantitative explanation of such optical properties as transition energy, oscillator strength, and the direction of transition moment, and for the interpretation of their several unique photochemical properties involving such acts as sensitizers in phototropism or in photo-induced reduction and polymerization. From these viewpoints several workers[3-5] have recently utilized semi-empirical self-consistent field (SCF) methods including electron repulsion explicitly for the calculation of the π-electronic structures of related compounds because preliminary computations using simple MO methods[6-8] have all been found to be inadequate for the above purposes except providing good reactivity indices for the predictions of the chemical and biological reactivities.

At first sight, it seems that in the process of the refinement of the SCF calculations for molecules of such complexity a difficulty lies as a result of the lack of reliable informations about the molecular geometry which seriously affects the semi-empirical evaluation for the two-center repulsion integrals and the core resonance integrals. In fact, different sets of the semi-empirical integrals have been chosen in the previous works according to the assumed molecular geometry and also the approximation model applied to the methyl group has been taken variously. Recently, in order to diminish the errors due to an assumed molecular geometry as far as possible the variable β-core procedure that recalculates the core resonance

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[1] Part I: M. Kamiya, *Nippon Kagaku Zasshi,* **90,** 769 (1969).

[2] Location: *Oshika 160, Shizuoka.*

[3] B. Grabe, *Biopolymers Symp.*, **1,** 283 (1964).

[4] J.L. Fox, K. Nishimoto, and L.S. Forster, *Biochim. Biophys. Acta,* **109,** 626 (1965); J.L. Fox, S.P. Laberge, K. Nishimoto, and L.S. Forster, *ibid.*, **136,** 544 (1967).

[5] W.E. Kurtin and P.-S. Song, *Photochem. Photobiol.*, **7,** 263 (1968); P.-S. Song, *J. Phys. Chem.*, **72,** 536 (1968).

[6] G. Karreman, *Bull. Math. Biophys.*, **23,** 35 (1961).

[7] K. Koziol, *Photochem. Photobiol.*, **5,** 41 (1961).

[8] B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, Inc., New York, 1963.

integrals after each SCF iteration on the basis of the assumed linear relation between the core resonance integral and corresponding bond order has been introduced; with the aid of this method, the Pariser-Parr-Pople SCF calculation has successfully been performed for isoalloxazine molecule without knowing its accurate molecular geometry.[4] This aspect of calculation is certainly of considerable significance when little structural information is available.

In the present paper, the Pariser-Parr-Pople SCF procedure embracing the variable β-core method has been extended to the calculation of the π-electronic structure of riboflavin together with invoking extensive configuration interactions and choosing an appropriate hyperconjugation model for the methyl group. The transition energy and oscillator strength, the direction of transition moment, the π-electron distributions of the lowest singlet and triplet excited states, and reactivity indices in the ground state have all been calculated, and discussions will be made by comparing these theoretical values with the experimental data.

Calculation Procedure

The Pariser-Parr-Pople method[9,10] is used for the calculation of the π-electronic state of the titled compound. The elements of F matrix are

$$F_{\mu\mu}=-I_{\mu}+\frac{1}{2}q_{\mu}\gamma_{\mu\mu}+\sum_{\nu\neq\mu}(q_{\nu}-n_{\nu})\gamma_{\mu\nu},$$

$$F_{\mu\nu}=\beta_{\mu\nu}-\frac{1}{2}P_{\mu\nu}\gamma_{\mu\nu},$$

where q_{μ} and $P_{\mu\nu}$ are the diagonal and off-diagonal elements, respectively, of the bond order-charge density matrix and n_{ν} is the number of electrons donated to the π-electron system by the νth atom. The one-center repulsion integral $\gamma_{\mu\mu}$ was usually estimated as I−A, where I is the ionization potential of atomic valence state and A is the electron affinity. In the present calculation the values of I and A were taken from the paper of Hinze and Jaffè.[11] The two-center repulsion integral $\gamma_{\mu\nu}$ was computed according to the Pariser-Parr approximation;

at the $R\leqq 2.8$ Å distances,

$$\gamma_{\mu\nu}=\frac{1}{2}(\gamma_{\mu\mu}+\gamma_{\nu\nu})+aR+bR^2 \text{ (eV)},$$

and at $R>2.8$ Å,

$$\gamma_{\mu\nu}=7.1975(1/\sqrt{R^2+1/4(R_{\mu}+R_{\nu})^2}+1/\sqrt{R^2+1/4(R_{\mu}-R_{\nu})^2} \text{ (eV)},$$

$$R_{\mu}=(4.597/Z^*)\times 10^{-8} \text{ cm},$$

where R is the internuclear distance, and a and b are parameters determined from the equation at $R>2.8$ Å when R is equal to 2.8 Å and 3.7 Å, respectively. Z^* is the effective nuclear charge.

The core resonance integral $\beta_{\mu\nu}$(ev) was iteratively estimated by the variable β-core method so as to lessen the errors due to the assumed molecular geometry according to the following equation:

$$\beta_{\text{C-C}}=-0.51\,\text{p}-1.84, \qquad \beta_{\text{C-N}}=-0.53\,\text{p}-2.02, \qquad \beta_{\text{C-O}}=-0.56\,\text{p}-2.20.$$

For the sake of simplicity the $\beta_{\text{C-N}_3}$ value of methyl group was fixed to −4.111 eV evaluated by the assumed relation $\beta_{\text{C-H}_3}/\beta=S_{\text{C-H}_3}/\text{S}$, where β is the core resonance integral over adjacent carbon atoms in benzen ring and S the overlap integral between two carbon 2pπ orbitals

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9) R. Pariser and R.G. Parr, *J. Chem. Phys.*, **21**, 466,767 (1953).
10) J.A. Pople, *Trans. Faraday Soc.*, **49**, 1375 (1953).
11) J. Hinze and H.H. Jaffé, *J. Am. Chem. Soc.*, **84**, 540 (1962).

separated by 1.39 Å. All bond lengths were assumed initially to be 1.39 Å except C–H_3 bond which was set at 1.10 Å according to the previous work[12] from which the parameters for the quasi-π orbital of H_3 in methyl group were adopted in reference to the successful SCF calculation of benzenium ion by taking hyperconjugation into account. The diketo structure was taken for riboflavin, and the numbering of the molecule is illustrated in Figure 1.

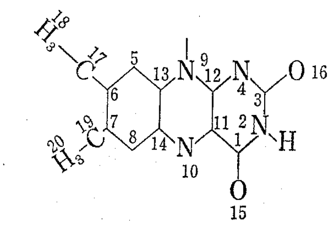

Fig. 1. The Numbering of the Titled Compound

The oscillator strength of a transition from the ground state to the excited state was calculated by using the following equation:

$$f=1.085\times 10^{-5}\sigma_{\mathrm{ob}}\boldsymbol{m}_{\mathrm{ob}}^2$$

where σ_{ob} expresses the wave number of the transition from the ground state (o) to the excited state (b) and m_{ob} is given by the equation

$$\boldsymbol{m}_{\mathrm{ob}}=\int\Psi_0^*\sum_i r_i\Psi_{\mathrm{b}}d\tau.$$

The excited configurations interacting with the ground configuration were chosen so as to involve the total 55 singly excited configurations which correspond to all one-electron excitations from the eleven occupied orbitals to the lowest-five vacant orbitals. In these cases the interconfigurational matrix elements were calculated according to the following equations:

$$({}^1\Phi_{i\to j}|\mathrm{H}|{}^1\Phi_{i\to j})=\varepsilon_j-\varepsilon_i-(J_{ij}-K_{ij})+K_{ij}$$

$$({}^1\Phi_{i\to k}|\mathrm{H}|{}^1\Phi_{j\to l})=2[ik|jl]-[ij|kl]$$

$$({}^3\Phi_{i\to j}|\mathrm{H}|{}^3\Phi_{i\to j})=\varepsilon_j-\varepsilon_i-(J_{ij}-K_{ij})-K_{ij}$$

$$({}^3\Phi_{i\to k}|\mathrm{H}|{}^3\Phi_{j\to l})=-[ij|kl]$$

$$[jl/ki]=\sum_{r,s}\sum C_r{}^jC_r{}^lC_s{}^kC_s{}^i\gamma_{rs}$$

where $C_j{}^i$ and ε_i denote the coefficient of the jth atomic orbital in the ith SCF molecular orbital and the orbital energy of ith SCF molecular orbital, respectively, and J_{ij} and K_{ij} denote Coulomb and exchange integrals over the ith and jth molecular orbitals, respectively.

All the calculations in this paper have been carried out on the HITAC 5020 computer at the Computation Center of Tokyo University. The semi-empirical parameters and effective nuclear charges utilized in the present calculation are collected in Table I.

Table I. The Parameters utilized in the Present Calculation

	I_μ (eV)	$\gamma_{\mu\mu}$ (eV)	$Z_\mu{}^*$
=C–	11.16	11.13	3.25
=O	17.28	15.27	4.55
=N–	14.12	12.34	3.90
$-\overset{\mid}{\mathrm{N}}-$	28.53	16.57	4.25
$-H_3$	10.256	9.326	2.83

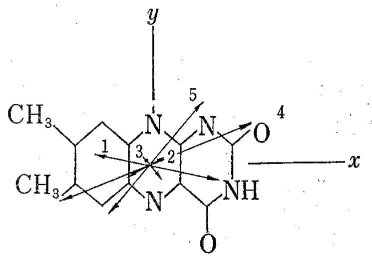

Fig. 2. Calculated Directions of the Transition Moments

The length of the moment is a relative intensity of the transition, and the numbering of the transition moments (1,2,···) is in pararell with the increase of the corresponding transition energies.

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[12] T. Morita, *Bull. Chem. Soc. Japan*, 33, 1486 (1960).

Results and Discussion

The calculated results of transition energies and oscillator strengths are presented in Table II with the experimental values. It may easily be known that the inclusion of configuration interactions as defined in the calculation procedure improves the calculated results through the general trend for lowering the calculated transition energies and oscillator strengths; the first transition energy is reduced by 0.41 eV in comparison with the initial value, but there still remains 0.46 eV discrepancy between the calculated and observed values of the first transition energy. In view of the complexity of the molecule itself even this result may be taken as rather possible and a similar tendency has also been observed in the other SCF–CI calculations, although different sets of semi-empirical integrals, especially concerning the value related to the methyl group, are employed and different types of configuration interaction methods are used. We feel that the improvement of the present result should be made in detail only after the completion of appropriate parametrizations for the following terms which have been neglected hitherto for their complexity; for example, the valence state ionization potential and electron affinity of the 9-nitrogen atom carrying the ribityl side-chain are likely to be altered considerably by the effect of polarization of the δ-core electrons and of the remarkable decrease of the π-electron density on this atom. Thus, it is desirable, first of all, that these values are carefully determined by adopting a variable core method[13)] so as to make allowance for this effect explicitly. Also, it seems that the validity of the assumed diketo structure has to be examined more critically. In this respect it is known that the calculated results based on a more unstable enol form produce not only little improvement for the calculated first transition energy but also much poorer results about other transitions.

Another problem is the choice of the semi-empirical parameters for the methyl group. According to the result of critical examinations[5)] on three models of the methyl group, inductive model, pseudo-heteroatom approximation, and group orbital hyperconjugation model, it is found that the third model employed in the present work seems to give the best results. Consequently, improvement of the calculated results may well be advanced on the basis of this model. Now, the calculated oscillator strength of the first transition band is higher than the experimental value, but this trend is more suppressed than the results of other calculations.

Meanwhile, the calculated results for other transitions are in a fairly good agreement with the experimental values; the second experimental band at 3.60 eV is successfully assigned to the calculated transition at 3.691 eV and, as will be mentioned later, this assignment can be confirmed by a rather good agreement between the calculated and obsrved results about the relative direction between the first and second transition moments. The third experimental band at 4.57 eV may well be related to either or both of the two calculated bands at 4.404 and 4.708 eV. However, the degeneracy of this band can not clearly be demonstrated by the data[5)] on the excitation polarization spectrum in this region, though the first and second bands are found to be probably non-degenerate. The weak transition at 4.083 eV is not actually observed but it shoud be noted here that the presence of a weak band corresponding to this is suggested on the basis of the polarization data[5)]

It may be of much interest to compare the theoretically determined direction of transition moments with the experimental results obtained from polarization spectrum. The calculated directions of the respective transition moments are shown in Figure 2, which indicates that the first absorption band polarizes close to the long-axis. The recent polarization data suggest that the angle between the transition moments of the first and second bands is about 49°[5)] or 30°[14)] and the angle between the second and third bands about 10°.[15)] The present

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[13)] For example, see R.D. Brown and M.L. Heffernan, *Trans. Faraday Soc.*, **54**, 757 (1958).

[14)] G. Weber, "Flavins and Flavoproteins," ed. E.C. Slater, Elsevier Publishing Co., 1966.

[15)] J.M. Lhoste, A. Haug, and P. Hemmerich, *Biochemistry*, **5**, 3290 (1966).

calculation gives about 27° to the former and about 6° to the latter angle, (In this case the third experimental band is assigned to the theoretical transition at 4.404 eV) and these results may be regarded as not so unreasonable in comparison with the experimental values.

As the triplet state is considered to play an essential role in the overall photochemistry of the molecule, extensive works[5,15–18] on phosphorescence in various matrices and SCF–MO–CI calculations of the triplet state energy have been made for the purpose of determining the species of the lowest triplet state. However, it seems at present that a conclusive remark as to whether this species is of the $^3(n,\pi^*)$ or $^3(\pi,\pi^*)$ type should be avoided until theoretical and experimental studies on this problem are sufficiently accumulated, although there is a recent study[17] on the phosphorescence polarization which claims that the lowest triplet of riboflavin is of $^3(\pi,\pi^*)$ type. In the present paper, the calculated results of the triplet state energy are collected in Table II, from which it is known that the calculated energy for the lowest $^3(\pi,\pi^*)$ is too low compared to experimental values for the lowest triplet (2.03—2.05 eV).

TABLE II. Comparison between the Theoretical and Experimental Spectra

Theoretical		Experimental[a] (eV)
With CI (eV)	Without CI (eV)	
3.280 (0.425)[b]	3.696 (11→12; 0.851)[c]	2.82 (0.24)
3.691 (0.147)	4.322 (10→12; 0.175)	3.60 (0.20)
4.083 (0.056)	4.482 (9→12; 0.339)	4.57
4.404 (0.768)	4.751 (11→13; 0.591)	5.55
4.708 (0.408)	5.244 (9→13; 0.760)	
5.295 (0.066)	5.579 (8→12; 0.095)	
5.450 (0.156)	5.952 (9→14; 0.214)	
5.635 (0.580)	6.020 (11→14; 0.044)	
5.656 (0.581)	6.207 (10→13; 0.096)	
6.090 (0.450)	6.280 (11→15; 0.030)	
Excitation energy of triplet state		
With CI (eV)	Without CI (eV)	
1.693	2.528	
2.431	3.519	
2.690	3.764	
3.296	3.943	
3.468	4.287	

a) In dioxane, taken from J. Koziol: *Photochem. Photobiol.*, **5**, 41 (1965) and Biochemist's Handbook, Ed by C. Long, 1961

Numerals in parenthesis are oscillator strengths evaluated from $\varepsilon_{\max}$ on the basis of the equation: $f=4.32\times10^{-19}\,\varepsilon_{\max}\,\Delta\nu$, where $\Delta\nu$ is the band width (in cm^{-1}) at half-maximum extinction.

b) Numerals in parenthesis are oscillator strengths.

c) Numerals in parenthesis are transition and oscillator strength, where the excitation energy $^{1,3}E_{i\to j}-E_0$ is obtained by the equation:

$$^{1,3}E_{i\to j}-E_0=\varepsilon_j-\varepsilon_i-(J_{ij}-K_{ij})\pm K_{ij}$$

The calculated results for the π-electron distributions of the lowest excited states (both singlet and triplet) have not previously been investigated in detail, although this is considered to be of much significance for a better understanding of several unique photochemical reactions of riboflavin. This result is shown in Table III, together with the π-electron distribution in the ground state. It is known, first of all, that at the 4-nitrogen atom a decrease of π-electron density and a concurrent increase of free valence proceed in the decreasing order of ground

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16) J. Posthuma and W. Berends, *Biochim. Biophys. Acta*, **122**, 422 (1966).
17) W.E. Kurtin and P.-S. Song, *Photochem. Photobiol.*, **9**, 127 (1969).
18) P.-S. Song and W.E. Kurtin, *J. Am. Chem. Soc.*, **89**, 4248 (1967).

Table III. The π-Electron Densities (q_r^{π}) and Free Valences (F_r^{π}) of Riboflavin in the Ground State (G) and the Lowest Singlet (S_1) and Triplet (T_1) Excited State

Atom	q_r^{π}			F_r^{π}		
	G	S_1	T_1	G	S_1	T_1
1	0.7762	0.8079	0.8139	0.1907	0.1716	0.1827
2	1.6061	1.5703	1.5810	0.7859	0.7867	0.7944
3	0.7221	0.7350	0.7326	0.1861	0.1744	0.1798
4	1.4636	1.2561	1.2421	0.7069	0.7211	0.7252
5	1.0578	1.0760	1.0961	0.4575	0.5078	0.5027
6	0.9435	0.9737	0.9787	0.2296	0.2926	0.2968
7	0.9909	0.9490	0.9428	0.2435	0.3001	0.3033
8	0.9439	1.0087	1.0266	0.4537	0.5064	0.5010
9	1.5077	1.4887	1.4843	0.7357	0.7877	0.7949
10	1.1488	1.3362	1.3295	0.4756	0.6460	0.6448
11	0.8763	0.9933	0.9847	0.3148	0.3515	0.3539
12	0.8990	0.9748	0.9764	0.1341	0.1697	0.1905
13	1.0926	1.0792	1.0629	0.1623	0.2195	0.2123
14	0.9796	0.9143	0.9104	0.1467	0.2088	0.2091
15	1.4966	1.5097	1.5168	0.9737	1.0125	1.0190
16	1.5081	1.3551	1.3484	0.9561	0.9950	0.9969
17	1.0126	1.0093	1.0090	0.5338	0.5260	0.5272
18	0.9779	0.9784	0.9801	0.7586	0.7698	0.7725
19	1.0092	1.0063	1.0059	0.5528	0.5449	0.5462
20	0.9876	0.9784	0.9777	0.7531	0.7617	0.7643

The calculations of q_r^{π} (S_1, T_1) and F_r^{π} (S_1, T_1) have been done according to the next equations:

$$q_r^{\pi}\ (S_1, T_1)=\sum_{i,j}(k_{i\to j}^{S_1,T_1})^2\times q_r^{\pi}\ (i\to j)$$

$$F_r^{\pi}\ (S_1, T_1)=\sqrt{3}-\sum_{l}\sum_{i,j}(k_{i\to j}^{S_1,T_1})^2\times p_{rl}^{\pi}(i\to j)$$

where q_r^{π} $(i\to j)$ and p_{rl}^{π} $(i\to j)$ are the π-electron density of the rth atom and the π-bond order between the rth and l th atoms, respectively, in the $i\to j$ singly excited configuration, and $k_{i\to j}^{S_1,T_1}$ is the coefficient of $i\to j$ singly excited configuration involved in the total wave functions of S_1 and T_1, respectively.

state, first excited singlet state, and first excited triplet state. Bearing in mind an accepted fact that in the ground state the 4-nitrogen acts as the acceptor of hydrogen and/or proton in the first step of reduction, it may be suggested from the above tendency that in the photoexcited state of riboflavin the possibility of the hydrogen-transfer mechanism is promoted if the reactive species has sufficiently prolonged life-time. Also, it is interesting that the order of the π-electron densities in the 4- and 10-nitrogens is reverse between the ground and first excited states ($q_{N(10)}^{\pi}<q_{N(4)}^{\pi}(G)\rightarrow q_{N(10)}^{\pi}>q_{N(4)}^{\pi}$ (S1,T1)), and this predicts a photo-induced change of the basicity because in the ground state the most basic center of the molecule is known to be the 4-nitrogen. In fact, such a photo-induced change of basicity has been evidenced from a lack of correspondence between the pH dependence of the fluorescence and the acid-base titration curves of riboflavin.[19]

It is also of much interest to correlate the photo-induced change of the π-electronic property of the 4-nitrogen with a mechanism proposed for the primary process of the anaerobic photobleaching of riboflavin which includes an intramolecular hydrogen-transfer from the ribityl side chain to the 4-nitrogen.[20] Namely, the above-mentioned photo-induced changes of the π-electron density and free valence of the 4-nitrogen seem to support a direct hydrogen-transfer since several workers have found effects suggesting that the photochemically reactive state is a triplet.[20,21] This finding is consistent with the experimental fact[22] that rate studies

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19) A. Albert, *Biochem. J.*, **54**, 646 (1953); P. Cerletti, *Anal. Chim. Acta*, **20**, 243 (1959).

20) M. Halwer, *J. Am. Chem. Soc.*, **73**, 4870 (1951); B. Holmstrom and G. Oster, *ibid.*, **83**, 1867 (1961).

21) R.H. Steele and L.C. Cusachs, *Nature*, **213**, 800 (1967).

22) W.M. Moore, J.T. Spence, F.A. Raymond, and S.D. Coulson, *J. Am. Chem. Soc.*, **85**, 3367 (1963).

on the anaerobic photolysis of the model flavins 9-(2′-hydroxyethyl)isoalloxazine and 9-(2′-hydroxyethyl-2′,2′-d_2)isoalloxazine show a kinetic isotope effect of 2.5 for the ratio k_H/k_D which agrees with that obtained for other photochemical hydrogen-abstraction reactions.

The reactivity indices calculated on the basis of SCF–MO's have scarcely been tested for the prediction and explanation of the reactivities of the titled compound. In this context the frontier electron densities $f^{(E)}$ and $f^{(N)}$ ($f_r^{(E)} \equiv 2 \times C_{r,}^{m^2}$ $m=$ highest occupied orbit., and $f_r^{(N)} \equiv 2 \times C_{r,}^{m+1^2}$ $m+1=$lowest vacant orbit.) were calculated and the result is presented in Table IV.

TABLE IV. The Frontier Electron Densities ($f_r^{(E)}$ and $f_r^{(N)}$) of Riboflavin

Atom	$f_r^{(E)}$	$f_r^{(N)}$	Atom	$f_r^{(E)}$	$f_r^{(N)}$
1	0.0000	0.0669	11	0.1206	0.3757
2	0.0140	0.0088	12	0.0000	0.1469
3	0.0028	0.0342	13	0.1409	0.0631
4	0.5937	0.0650	14	0.1818	0.0015
5	0.0144	0.0808	15	0.0106	0.0959
6	0.0812	0.1475	16	0.3872	0.0493
7	0.1369	0.0099	17	0.0034	0.0015
8	0.0002	0.1786	18	0.0179	0.0242
9	0.2529	0.1986	19	0.0037	0.0005
10	0.0147	0.4495	20	0.0228	0.0015

TABLE V. Wave Functions of the Lowest Singlet and Triplet Excited States

$$\Psi_{(S_1)} = 0.8799\,{}^1\Phi_{11\to12} + 0.1341\,{}^1\Phi_{11\to13} + 0.2675\,{}^1\Phi_{10\to12} - 0.1435\,{}^1\Phi_{9\to12} + 0.2241\,{}^1\Phi_{9\to13} + 0.1463\,{}^1\Phi_{8\to14}$$
$$\Psi_{(T_1)} = 0.8536\,{}^3\Phi_{11\to12} - 0.1391\,{}^3\Phi_{11\to13} - 0.1945\,{}^3\Phi_{11\to14} - 0.2223\,{}^3\Phi_{11\to15} + 0.2319\,{}^3\Phi_{10\to12}$$

Only the coefficients of important configurations are given.

It is known from this that the most active positions for electrophilic and nucleophilic reactions are the 4- and 10-nitrogens, respectively. In other words, the first step of reduction of flavin coenzyme may be due to an ionic process as usually accepted for biological oxidation and reduction.

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