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Electronic Structure of Flavins. Inclusion of Methyl Groups in Molecular Orbital Treatments of Flavins

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Abstract Various MO methods with differing degrees of sophistication are shown to yield qualitatively consistent results for methyl isoalloxazines. However, with crude methods such as the HMO and w-technique, the choice of Coulomb and resonance integralsis critical, in contrast with simpler molecular systems. The empirical value of $w=0.5$ appears to be more reasonable than 1.4.

Methyl groups in these flavins are best treated by the group orbital approximation. The pseudo-heteroatom approximation overestimates methyl hyperconfiguration with the Pariser-Parr-Pople SCF MO method. Singlet $\pi\rightarrow\pi^*$ transition energies are calculated by the P-P-P method and agree reasonably with the experimental values. 2- and 4-Thioisoalloxazine analogs are also treated. Reactivity indices of the flavin molecule are presented, including superdelocalizability, frontier orbital and radical densities. Various aspects of the applications of these indices to some chemical and biological reactivities of flavins are briefly discussed. The effects of the methyl groups on dipolemoments, ionization potentials, electron affinities, and spectra are described in detail.

Introduction

Flavins have frequently been treated by the Hückel MO and SCF MO theories.[1-6] However, the 7, 8-methyl groups of the isoalloxazine molecule are not included in these treatments except in the HMO(group orbital approximation)[7] by Pullman and Pullman[1] and the w-method (pseudo-heteroatom approximation)[8,9] by the author.[3]

The objective of the present study was twofold. First, we have critically examined three methods of treating methyl groups in isoalloxazines. These include the inductive model (I),[10,11] the pseudo-heteroatom approximation(H)[8,9] and the group orbital hyperconjugation model (G)[7]. In examining these models, we have also employed the P-P-P SCF MO with configuration interaction within the restricted Hartree-Fock framework. It is hoped that results will aid in understanding the contribution of the methyl group to the chemical and biological specificities of other biomolecular systems.

The alkyl group at N_{10} was not included in the present work, since this group will not contribute significantly to the π-conjugation of the flavin ring via the pyrrolic nitrogen. In support of this ascertion, for example, the alkyl

substituents on N_{10} (N_3) have only a small influence on the absorption spectra.[12] However, this group does affect the chemical reactivity of the molecule, as will be mentioned later.

The second objective was to show the magnitude and the significance of the methyl contribution to the electronic structure, spectra, reactivity and biological specificity of flavin analogs. Since the methyl groups affect the electronic properties of the molecule, and the 8-methyl group is reactive,[13,14] we suggest that the inclusion of the methyl groups is essential for the interpretation of the MO results with respect to the chemical and biological characteristics of flavins. Additional data pertinent to the present attempt at including the methyl groups in the flavin ring are as follows:

(1) Methyl substitution at position 6, 7, and 8 results in a bathochromic shift in one or two long wavelength absorption bands of isoalloxazine.[12,15,16,17]

(2) The number and position of the methyl substituent critically affect the oxidation-reduction potentials of flavins.[18] Since the biological function of flavins is in the electron transfer process, it is important to examine the contribution of the methyl substitution to the oxidation-reduction potentials.

(3) An uneven spin density distribution is observed for positions 7 and 8 with the methyl groups,[19] indicating a different magnitude of the effect of the methyl substitution upon the electronic structure of the open-shell system. This may be significant in the photochemical and oxidative reactivity of flavins. For this reason, open-shell computations of flavins are in progress, and preliminary results have been presented recently.[20]

(4) The methyl groups at positions 7 and 8 are essential for biological activity(see Ref. 18 for more details and references therein). It has been emphasized that the methyl group at position 8 is biochemically important,[21] and covalently bound flavins of certain flaveonzymes involve 8-methylgroup.

We have undertaken a detailed study of the effects of methyl substitution on the electronic structures of biomolecules, and the part of the results on flavins are described in the present paper.

Methods

Since the theories and computational procedures of the various MO methods employed are described in detail elsewhere,[9,22-24] we will only comment on the choice of empirical and

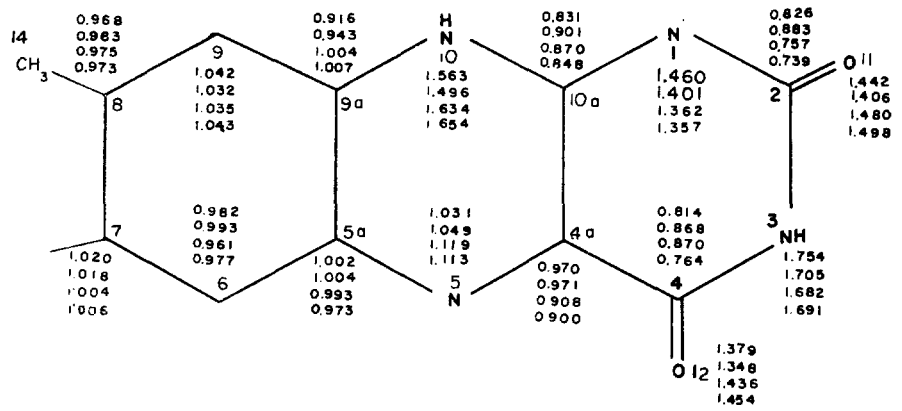

Fig. 1. π-Electron density distribution in isoalloxazine calculated by different methods: From top to bottom; HMO, w-SCF (0.5), P-P-P (I_s), and P-P-P (I_n).

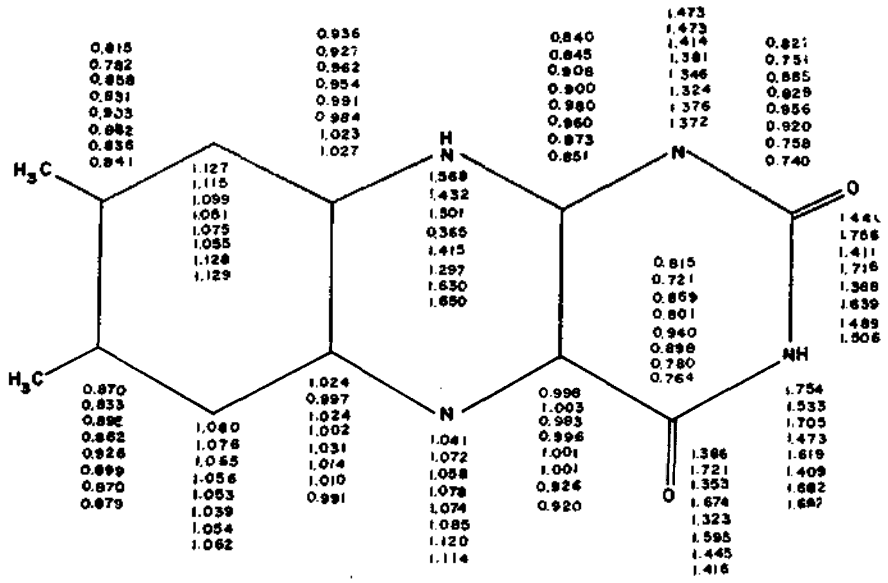

Fig. 2. π-Electron density distribution in 7,8-dimethyl-isoalloxazine (I-model) calculated by different methods: From topto bottom: HMO (I_h), HMO (I_h'), ω=0.5 (I_h), ω=0.5 (I_h'), ω=1.4 (I_h), ω=1.4 (I_h'), P-P-P (I_1), and P-P-P (I_2).

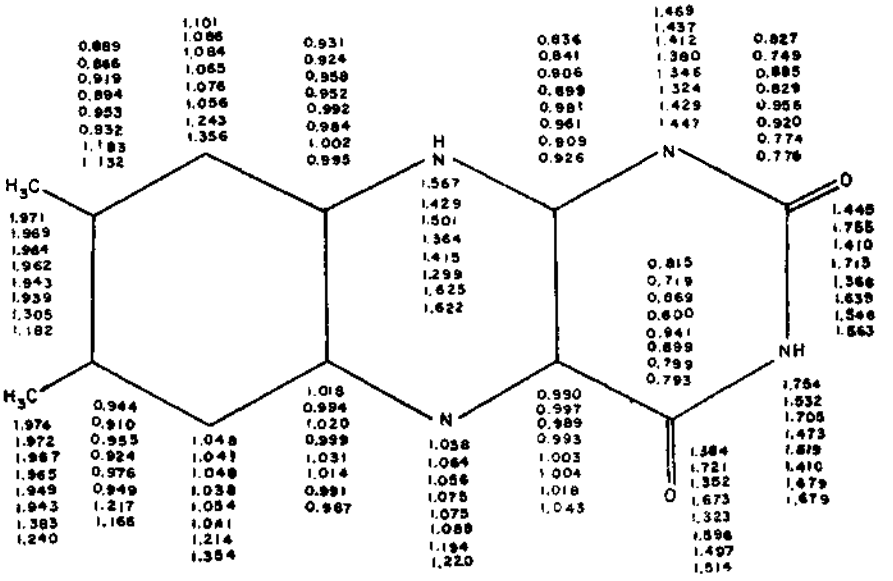

Fig. 3. π-Electron density distribution in 7,8-dim ethyl-isoalloxazine (H-model) calculated by different methods: From top to bottom: HMO (H_h), HMO (H_h'), ω=0.5 (H_h), ω=0.5 (H_h'), ω=1.4 (H_h), ω=1.4 (H_h'), P-P-P (H_1), and P-P-P (H_2).

semiempirical integrals in each method.

(1) HMO. Two sets of parameters were used. The first set was identical with that of Ref. 22, and the second set(I_h', H_h', G_h') was one which we had previously used[25] and which was recommended by the Kyoto laboratory.[26] The latter

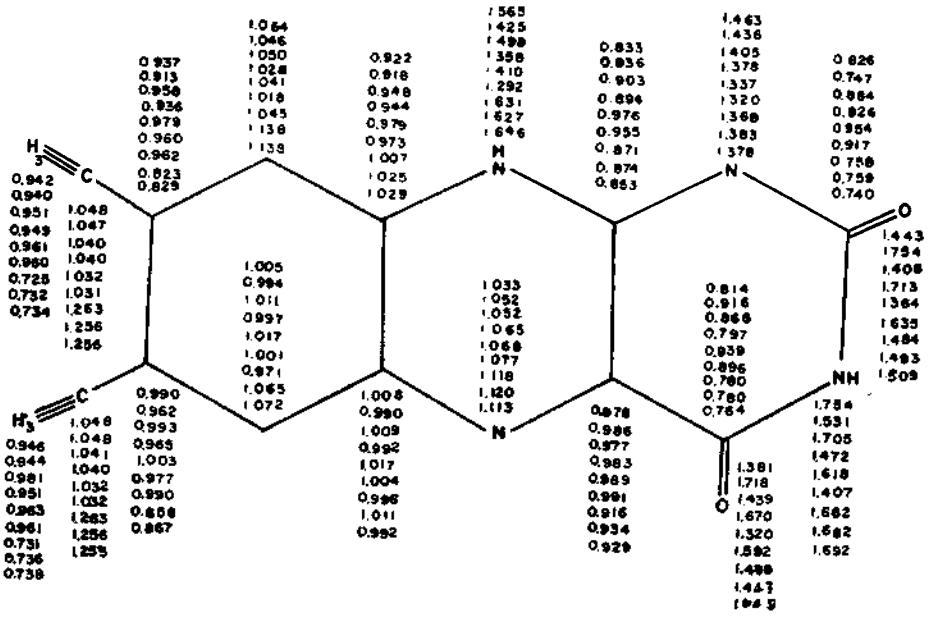

Fig. 4. π-Electron density distribution in 7, 8-dimethyl-isoalloxazine (*G*-model) calculated by different methods: From top to bottom: HMO (G_h), HMO (G_h'), ω=0.5 (G_h), ω=0.5 (G_h') ω=1.4 (G_h) ω=1.4 (G_h'), P-P-P (G_1), andP-P-P (G_2), and P-P-P (G_3).

set was compiled fromvarious sources.

(2) *w*-SCF HMO. An empirical introduction of the electron interaction into the HMO scheme can be made in terms of the *w*-iteration technique. The procedure is to iterate starting with the HMO parameters followed by each new set of α until α converges: $\alpha_r=\alpha_c+(1-P_{rr})\omega\beta_c$, where $\omega=-0.25$ $(rr|rr)/\beta_c$ and P_{rr} is a diagonal element of the bond-order matrix. $(rr|rr)$ is the usual one-center electron repulsion integral.[27] Both ω=0.5[27] and 1.4[9] were used in the present work.

(3) P-P-P SCFMO. The usual Goeppert-Meyer-Sklar(GMS) and zerodifferential overlap (ZDO) approximations,[28] semiempirical evaluation of integrals,[23,26,29,30] and neglect of penetration integrals(NPI)[28] were assumed. The values of the ionization potentials, one- and two-center electron repulsion integrals, and neighboring β_{rs} terms were the same as those previously used in our laboratory.[25,30] The integral values for the methyl groups were adopted from refs. 31-33.

In our attempt to refine the P-P-P MO results, all non-neighbor β_{rr} terms (I_n, I_2, H_2, G_3) were also included in the evaluation of the off-diagonal elements (F_{rs}) of the Fock matrix, and the results are to be compared with those of the neighbor β_{rs}-only calculations (I_s, I_1, H_1, G_1). Non-neighbor β_{rs} terms were estimated from the well-known proportionality relation between the Slater atomic orbital overlap integral and β_{rs}.[28]

The configuration interaction (CI) matrices were constructed with a limited number of singly-excited configurations, usually 25 configurations. Since the inclusion of all singly- and double-excited configurations for flavins is computationally impractical, the relative invariance (±0.01 eV) of the lowest excited singlet state

energy was taken as a critertion to limit the order of the CI matrix. This criterion was met in our calculations within 18-22 configurations. All computations were performed on IBM 7040 and IBM 360/50.

Results and Discussion

1. Electron Densities and Geometry. Electron densities (P_{rr}) for isoalloxazine (*Fig.* 1) and 7, 8-dimethyl isoalloxazine molecules (*Figs.* 2, 3 and 4) are shown. * The HMO data (I_h and G_h) is in satisfactory agreement with the previous, [22] within rounding-off and truncation errors. Comparison of *Fig.* 2, 3, and 4 with *Fig.* 1 suggests the following observations:

(a) There is a quantitative influence of the methyl groups on the electron density distribution throughout the molecule, particularly in the vicinity of the methyl groups. This indicates some hyperconjugative contribution of the methyl groups to the ring system. This effect is particularly pronounced in *Fig.* 3. However, it will be shown later that the pseudo-heteroatom model overestimates hyperconjugation.

(b) Despite the quantitative effect noted in (a), the general charge distribution patterns are not altered. For example, the different sets of parameters, methods, and methyl group treatments (*Figs.* 2-4) consistently yield a significantly higher electron density for N_1 than for N_5, for O_{11} than for O_{12}, and for N_3 than for N_{10}. Therefore, one may predict, for example, that N_1 must have pK_a greater than that of N_5. Experimentally, flavins show two pK_a's, near zero and 10[16]. The relative basicity of N_1 and N_5 has been theoretically predicted to reverse upon excitation to the lowest singlet ortriplet state. [20,34]

(c) in the inductive treatment (I) of the molecule (*Fig.* 2), a qualitative agreement among the P_{rr} values obtained from different sets of parameters and methods can be seen. However, it is also clear that the values at heteronuclei calculated using model I_h show better agreement than model I_h' with the P-P-P results, For example, while P_{rr} at O_{11} and O_{12}, calculated with the parameter set of I_h, are significantly higher than those obtained by other methods, P_{rr} at N_3 and N_{10} are shown to be considerably lower. These discrepancies are due to the different Coulomb (C=O) and resonance (C−NH−) integrals in the parameter set of Ref. 26.

(d) The charge distribution is not significantly affected by the inclusion of non-neighbor β_{rs} terms(in G_3 calculations). In fact, the difference between models G_1 and G_2 is larger than between models G_2 and G_3, owing to the difference in the semiempirical integrals of the former models.

Table 1 lists the selected bond distances calculated from the bond orders according to the Nishimoto-Forster formulas. [6] Bonds listed are selected from heteronuclear and long C-C bond types. The predicted bond distances in *Table* 1 are, in general, consistent with the crystallographic data[35]. The bond distance(R) for C-CH_3 was calculated from an approximate formula:

$$R_{C\text{-}CH_3}=1.533-0.18P_{C\text{-}CH_3}=1.52\ \text{Å}$$

where the empirical constant (1.553) was determined by knowing $P_{C\text{-}CH_3}=0.183$[36]and the observed value of 1.52 Å for toluene.

A close examination of *Table* 1 reveals the following points: (a) Within a given method, HMO or ω-technique, different approximations for the methyl groups do not markedly affect the molecular geometry. However, the P-P-P results show a noticeable dependence upon the methyl group approximations used, especially

*Detailed molecular orbital diagrams are available upon request. See appendix for additional *Figures* (*Figs.* 9, 10, 11, 12).

Table 1. The calculated distances (Å) of selected bonds.

Method	Model	N_1-C_{10a}	C_2-O_{11}	C_4-C_{4a}	C_4-C_{12}	$C_{4a}-C_{10a}$	$C_{4a}-N_5$	C_7-C_8	$N_{10}-C_{9a}$	C_7-C_{13}	C_8-C_{14}*
HMO	I_h	1.340	1.267	1.460	1.260	1.431	1.322	1.413	1.369		
	I_h'	1.353	1.312	1.442	1.305	1.428	1.334	1.419	1.357		
	H_h	1.339	1.267	1.460	1.260	1.431	1.321	1.412	1.369	1.521	1.521
	H_h'	1.353	1.307	1.443	1.305	1.429	1.333	1.417	1.357	1.522	1.520
	G_h	1.339	1.266	1.461	1.260	1.432	1.320	1.411	1.368	1.520	1.519
	G_h'	1.353	1.311	1.444	1.304	1.430	1.331	1.415	1.357	1.519	1.519
$\omega=0.5$	I_h	1.337	1.266	1.460	1.260	1.432	1.321	1.411	1.364		
	I_h'	1.351	1.307	1.443	1.300	1.429	1.333	1.416	1.353		
	H_h	1.336	1.266	1.460	1.260	1.432	1.321	1.412	1.364	1.520	1.518
	H_h'	1.350	1.307	1.443	1.300	1.430	1.332	1.417	1.353	1.518	1.516
	G_h	1.335	1.266	1.461	1.260	1.433	1.319	1.411	1.363	1.520	1.519
	G_h'	1.350	1.307	1.444	1.299	1.430	1.330	1.415	1.353	1.519	1.519
$\omega=1.4$	I_h	1.333	1.267	1.461	1.262	1.433	1.320	1.409	1.358		
	I_h'	1.346	1.300	1.445	1.294	1.431	1.330	1.413	1.350		
	H_h	1.333	1.267	1.460	1.262	1.433	1.321	1.414	1.358	1.512	1.510
	H_h'	1.346	1.300	1.445	1.294	1.431	1.331	1.418	1.350	1.510	1.508
	G_h	1.331	1.266	1.461	1.262	1.434	1.319	1.411	1.357	1.520	1.521
	G_h'	1.345	1.299	1.446	1.293	1.432	1.329	1.414	1.350	1.519	1.519
PPP	I_1	1.324	1.270	1.463	1.268	1.447	1.310	1.409	1.379		
	I_2	1.323	1.271	1.461	1.269	1.447	1.310	1.407	1.384		
	H_1	1.338	1.278	1.453	1.274	1.429	1.335	1.448	1.371	1.437	1.429
	H_2	1.343	1.280	1.451	1.276	1.423	1.342	1.468	1.368	1.414	1.407
	G_1	1.322	1.270	1.463	1.268	1.448	1.309	1.410	1.379	1.506	1.506
	G_2	1.324	1.271	1.462	1.268	1.446	1.311	1.414	1.379	1.520	1.520
	G_3	1.324	1.271	1.461	1.269	1.446	1.311	1.412	1.384	1.520	1.520

*See text.

for the C-CH_3 bonds. In other words, the difference in geometry arising from different methyl group treatments does not appear with more crude methods. The P-P-P results (I_r model) for isoalloxazine are in general agreement with those of Forster et al., who employed the variable-β procedure.

(b) HMO, ω-SCF HMO with the Pullman parameter set,[22] and the P-P-P methods give consistent results, but those HMO and ω-MO methods with the other set of paramethers[26] yield qualitatively unsatisfactory geometries, especially in the right half of the molecule including CO and CN groups.

(c) The pseudo-heteroatom (H) treatment clearly overestimates the hyperconjugation, shortening the C-CH_3 bonds. This is particularly apparent with the P-P-P method. Consequently, the H-model tends to lengthen the C-C bonds next to the methyl groups. Interestingly, the C-CH_3 distances decrease as a function of diferent MO methods, in order of HMO, $\omega=1.4$, and P-P-P methods.

(d) The C_4-C_{4a} and C_{4a}-C_{10a} distances are found to be longer than the average C-C bond distances. However, these are not usually long. For example, both the observed[35] and calculated bond distances are of a comparable magnitude.

Our P-P-P results on P_{rr} and P_{rs} slightly disagree with those reported by Grabe[4] due to the different values of the integrals used. The use of lower values of the atomic ionization potentials than those of one-center electron repulsion integrals by Grabe seem to cause somewhat unreasonable electron distributions(see also Ref. 30.). However, our results are in a qualitative agreement with her previous calculations. These can not be compared directly with our data, since she calculated only a part of the isoalloxazine molecule.

2. π-Dipole Moments (μ_π). From the comparison of different methyl treatments in Section 1, it is possible to conclude that models *I* and *G* are more satisfactory than the *H*-model. We will further demonstrate this point later. We now extend our discussion to the effects of the methyl substitution on molecular properties which are important for understanding the chemical and biological specificities of flavins. *Fig.* 5 shows the μ_π as calculated by the P-P-P method. Since we have shown in Section 1 that more crude methods are not sensitive enough to discriminate the different methyl group approximations, only the P-P-P results are presented. It can be observed from *Fig.* 5 that the *H*-model yields an unreasonably large dipole moment due to the extensive charge contribution of the methyl groups to the ring(see also *Table* 1). The *G*-model(except for G_1) results in a some what higher μ_π than the *I*-model. In either case, however, the methylation of the isoalloxazine is accompanied by an increase in μ_π (*Fig.* 6), consistent with observations. Which of the models (*I* and *G*) more adequately represents the magnitude of the increase in μ_π is debatable. However, we noticed that the same relative trend as in *Fig.* 6 (based on the G_3-model) is reproduced with models *I*, G_1 and G_2. *Fig.* 6 includes data for other analogs to compare the effect of different methyl substitution (*i.e.*,

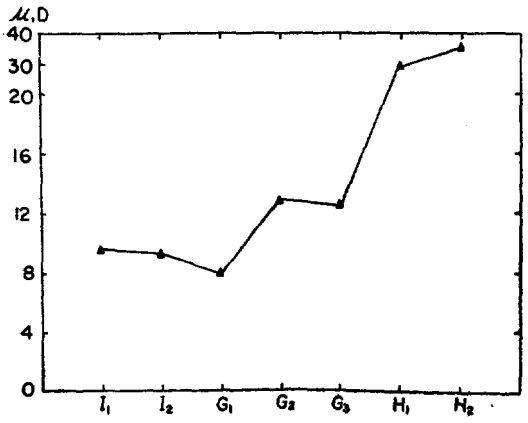

Fig. 5. π-Dipole moments of 7,8-dimethyl-isoalloxazine calculated by different methyl group approximations (P-P-P). Notice change in scale of the ordinate.

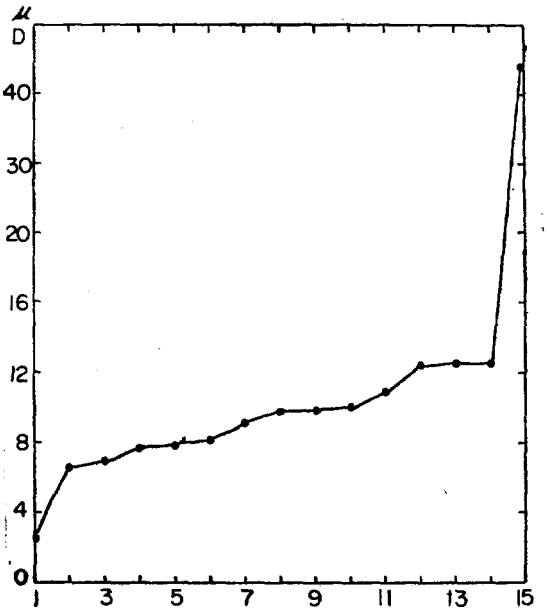

Fig. 6. π-Dipole moments of methyl flavin analogs (abscissa) calculated by the P--P-P G_3 method: 1; alloxazine, 2; isoalloxazine, 3; 6-methyl-, 4; lueco-7,8-dimethyl-, 5; 9-methyl-, 6; 7,8-dimethyl-alloxazine (lumichrome), 7; 7-methyl-, 8; 8-methyl-, 9; 6,7-dimethyl-, 10; 7,9-dimethyl-, 11; 8,9-dimethyl-, 12; 7,8-dimethyl-, 13; 6,7,8-trimethyl-, 14; 4-thio-7,8-dimethyl-, and 15; 2-thio-7,8-dimethyl-.

position and number of the substituent).

It can be seen that 7, 8-dimethyl-isoalloxazine, a biologically active form, has the largest μ_π among the analogs, except for 6, 7, 8-trimethyl- and 7, 8-dimethylthioisoalloxazines. The dipole moment for the former is also twice that of isoalloxazine, a biologically inactive form.[18] Apparently, 4-thio-substitution does not affect the dipole moment of the parent flavin as markedly as the 2-thiosubstitution. While the calculated π-moments are not directly comparable with the observed value, the dipole moment for lumiflavin(7, 8-dimethyl-10-methyl-isoalloxazine) has been observed to be roughly 16 D,[37] indicating considerable polarization of the σ- and π-systems due to the heteroatoms and methyl groups in flavins. The increase in dipole moment due to the methyl substitutions has been discussed frequently.[38] For isoalloxazine itself, the total dipole moment has been calculated tobe 5.5 D[20], which is not too different from the π-moment alone (*Fig.* 6).

3. **Ionization Potentials.** *Fig.* 7 shows the Koopmans' theorem[39] ionization potentials (I_p) for different methyl flavins. All four methods correctly predict a lower I_p for the leucoflavins than for the oxidized ones, as is to be expected from the biological electron-donor property of leucoflavins with negative oxidation potentials. *Table* 2 clearly shows the lowering of the I_p

Table 2. Ionization potential and electron affinity(A) of 7, 8-dimethylisoalloxazine

Method	Model	I_p	A	Remark
HMO	I_h	0.198 eV	0.380*	Isoalloxazine, −LEMO=0.329
	I_h'	8.639	0.196	
	H_h	8.238	0.363	
	H_h'	8.676	0.179	
	G_h	8.036	0.339	
	G_h'	8.753	0.154	
ω=0.5	I_h	7.879	0.425	0.373
	I_h'	8.432	0.233	
	H_h	7.893	0.412	
	H_h'	8.442	0.222	
	G_h	7.952	0.384	
	G_h'	8.517	0.193	
ω=1.4	I_h	7.331	0.516	0.460
	I_h'	7.969	0.324	
	H_h	7.317	0.517	
	H_h'	7.941	0.328	
	G_h	7.394	0.472	
	G_h'	8.029	0.283	
P-P-P	I_1	9.218	3.381	I_p ; I_s : 9.701 eV
	I_2	8.732	3.087	I_n : 9.256
	H_1	5.771	2.139	A ; I_t : 3.714
	H_2	5.632	1.360	I_n : 3.392
	G_2	8.907	3.216	
	G_3	8.428	2.933	

*-LEMO as a relative A scale for HMO and ω-MO results.

upon methyl substitution of isoalloxazine. This is reflected consistently by all methods and different HMO parameters, although quantitative differences are observable. Since the methods used are approximate, only relative values of I_p are meaningful in this case. For example, the neglect of penetration in the P-P-P method obscures the values of Koopmans' theorem I_p. However, two results in *Table* 2 are note worthy. First, crude MO methods (HMO, ω-HMO) are relatively insensitive to the different methyl group approximations. Thus, they are inadequate for examining the validity of the different methyl group treatments. Second, the P-P-P *H*-model results are obviously unacceptable. In fact, the *H*-model I_p's are close to those of leucoflavins shown in *Fig.* 7. It can also be concluded that the *I* and *G* models are more or less equivalent, at least semiquantitatively. In this connection, Flurry has recently shown the effect of the methyl group on I_p in terms of the inductive model.[40] Both calculations[40] and experiments[41] show a decrease of the I_p of benzene by about 0.5-0.7 eV upon mono-or dimethyl substitutions. This is satisfactorily reflected in *Table* 2 and *Fig.* 7.

Because of the biological significance of flavins as electron-donor-acceptor catalysts and the requirement of the 7,8-methyl groups for full biological potency, we now examine the effect of the methyl substituents in more detail. For this purpose, the data in *Fig.* 7 were obtained from the G_3-model calculations. However, the same set of data has been calculated by *I*-models for a few derivatives, showing a practically identical pattern.

It can be seen that crude methods are not able to produce the variation of I_p within each group of oxidized and reduced(leuco) flavins. Experimentally, the number and position of the methyl substituent inaromatic molecules affect I_p to a significant extent.[41] For example, the extent

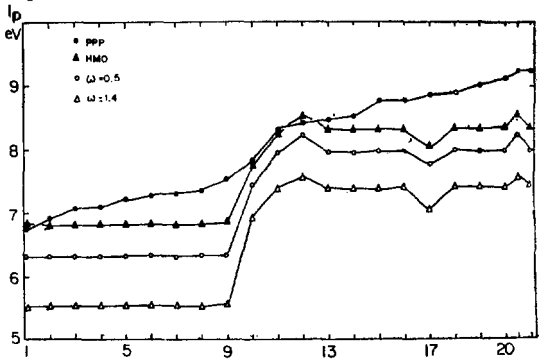

Fig. 7. Ionization potentials of methyl flavin analogs (abscissa). $\alpha=-7.06$ eV and $\beta=-2.49$eV[26] were used to evaluate I_p's from HMO(G_h) and ω-SCF MO (G_h) procedures. The G_3-model was used in the P-P-P MO: 1; leuco-6,7,8-trimethyl, 2; leuco-7,8,-dimethyl-, 3; leuco-8,9-dimethyl-, 4; leuco-7,9-dimethyl-, 5; leuco-8-methyl-, 6; leuco-7-methyl-, 7; leuco-6-methyl-, 8; leuco-9-methyl-, 9; leuco-isoalloxazine, 10; 2-thio-7,8-dimethyl-, 11; 6,7,8-trimethyl-, 12; 7,8-dimethyl-alloxazine (lumichrome), 13; 7,8-dimethyl-, 14; 7,9-dimethyl-, 15;8,9-dimethyl-, 16; 7-methyl-, 17; 4-thio-7,8-dimethyl-, 18; 8-methyl-, 19; 9-methyl-, 20; 6-methyl-, 21; alloxazine, and 22; isoalloxazine.

of the lowering of I_p by methyl substituents for toluene, naphthalene, pyridine, and aniline depends very much upon the number and position of the substituent.[41] This well-known behavior is detectable with the data obtained from the P-P-P calculations (*Fig.* 7) It is therefore interesting to note that leuco 7,8-dimethyl-isoalloxazine, a biologically important form, is predicted to be a better electron donor(*i. e.*, lower I_p) than the others except for the leuco-6,7,8-trimethyl-isoalloxazine. This remarkable electron donor property predicted by the P-P-P MO appears to be mainly due to the substitution at position 8, since leuco 8,9-and leuco 8-methyl analogs have higher I_p's than leuco 7,9-and leuco 7- (or 6- and 9-) methyl analogs, respectively. The same trend is observable in the case of oxidized analogs. These results are consistent with the idea that 7,8-dimethyl-fiavin should be a good electron acceptor, while being

a moderate electron-donor.[22,42]

4. Electron Affinities. It is well known that the I_p and electron affinity (A) correlate with the electron-donor and electron-acceptor capacity of a series of organic compounds.[22,42] *Fig.* 8 shows the Koopmans' theorem electron affinities (from P-P-P MO) and the negative of the LEMO energy coefficients for various flavin analogs. It can be seen that the results of the different MO methods are rather similar to those shown in *Fig.* 7, as expected. Thus, no detailed discussion is made in this section. However, it appears that 7,8-dimethyl-isoalloxazine is a moderate electron-acceptor with A lower than thio- and monomethyl flavins but higher than trimethyl-, leuco flavins and lumichrome. It is apparent from *Fig.* 7 that leuco isoalloxazine and leuco 7,8-dimethyl-isoalloxazine should be good electron donors. From *Fig.* 8, it can also be noted that isoalloxazines are better electron-acceptors than alloxazines.

Although experimental electron affinities are not available for comparison, the values from the P-P-P calculations are certainly of the expected magnitude. For a limited number of aromatic compounds, a good linear correlation usually exists between the negative of the LEMO energy coefficients and the observed A's.[22] *Table* 2 shows the dependence of the numerical values of the A's upon parameters and methyl group models. The relative trend from the different methyl models is reproduced consistently within the same set of HMO parameters. Crude methods again show their insensitivity toward the methyl approximations, while the P-P-P calculations reveal the inadequacy of the H-model. Namely, I_p and A from the H-model calculations are too low, probably due to the overestimation of the methyl hyperconjugation to the ring system. It is also noteworthy that the inclusion of all non-neighbor β_{rs} terms (I_2 and

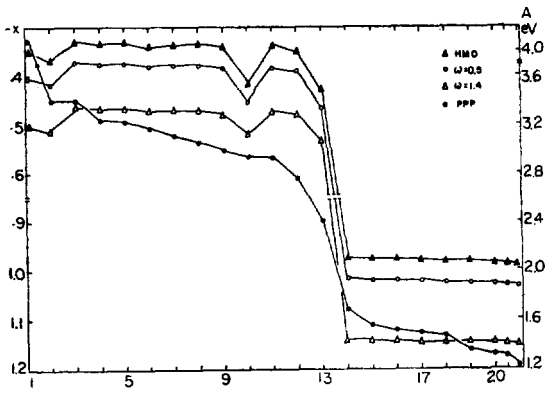

Fig. 8. The negative of the LEMO eigenvalues (X) (left ordinate) and electron affinities (A, right ordinate) of methyl flavin analogs (abscissa)calculated by G_h- (HMO, ω) and G_s- (P-P-P) models, respectively: 1; 2-thio-7,8-dimethyl-, 2; 4-thio-7,8-dimethyl-, 3; isoalloxazine, 4; 9-methyl-, 5; 7-methyl-, 6; 6-methyl-, 7; 8-methyl-, 8; 7,9-dimethyl-, 9; 8,9-dimethyl-, 10; alloxazine, 11; 7,8-dimethyl-, 12; 6,7,8-dimethyl-, 12; 6,7,8-trimethyl-, 13; 7,8-dimethyl- alloxazine(lumichrome), 14; leuco-isoalloxazine, 15; leuco-6-methyl-, 16; leuco-9-methyl-, 17; leuco-8-methyl-, 18; leuco-7-methyl-, 19; leuco-8,9-dimethyl-, 20; leuco-7,9-dimethyl-, 21; leuco-7,8-dimethyl-, and 22; leuco-6,7,8-trimethyl-

G_3) in the P-P-P MO procedure tends to yield lower I_p and A values than the I_1 and G_2 models. A similar pattern was observed with leuco 7,8-dimethyl-isoalloxazine, lumichrome, and thioisoalloxazines.

5. Reactivity. Super delocalizability for nucleophilic attack (SDN) and frontier orbital densities(FOD, in parentheses) at selected positions of the 7,8-dimethyl-isoalloxazine molecule are presented in *Table* 3. *Table* 4 shows superdelocalizabilities for radical attack (SDR) and frontierradical densities (FRD). These tablesare presented here to serve as an interpretative tool for reactivities of the basic flavin moiety. For example, C_4 should be slightly more reactive electronically toward a nucleophilic reagent such as the amino group than C_2. Neims et al. proposed a Schiff base formation at C_2 with the

Table 3. SDN and FOD (parenthesized) at selected positions of the flavin nucleus.

Method	atom	N_1	C_2	C_4	C_{4a}	N_5	C_{5a}	C_6	C_9	C_{9a}	C_{10a}
HMO	I_k	0.448	0.708	0.805	1.122	1.790	0.650	0.934	0.692	0.974	1.096
		(0.058)	(0.023)	(0.057)	(0.267)	(0.599)	(0.007)	(0.153)	(0.034)	(0.122)	(0.115)
	H_k	0.466	0.711	0.811	1.176	1.849	0.657	1.025	0.754	0.982	1.116
		(0.062)	(0.023)	(0.056)	(0.275)	(0.588)	(0.004)	(0.171)	(0.047)	(0.110)	(0.116)
	G_k	0.493	0.716	0.821	1.263	1.936	0.674	1.155	0.855	1.003	0.734
		(0.066)	(0.022)	(0.055)	(0.286)	(0.571)	(0.001)	(0.192)	(0.069)	(0.096)	(0.115)
$\omega=0.5$	I_k	0.468	0.647	0.730	1.088	1.569	0.641	0.910	0.719	0.892	0.950
		(0.061)	(0.021)	(0.052)	(0.289)	(0.573)	(0.004)	(0.163)	(0.049)	(0.106)	(0.088)
	H_k	0.479	0.649	0.733	1.118	1.597	0.649	0.963	0.761	0.900	0.959
		(0.063)	(0.021)	(0.051)	(0.292)	(0.562)	(0.002)	(0.174)	(0.059)	(0.098)	(0.088)
	G_k	0.508	0.654	0.741	1.202	1.673	0.670	1.088	0.864	0.926	0.981
		(0.068)	(0.021)	(0.050)	(0.304)	(0.542)	(0.000)	(0.197)	(0.085)	(0.084)	(0.087)
$\omega=1.4$	I_k	0.464	0.554	0.609	0.997	1.269	0.618	0.861	0.730	0.786	0.766
		(0.059)	(0.017)	(0.041)	(0.324)	(0.534)	(0.001)	(0.184)	(0.076)	(0.084)	(0.055)
	H_k	0.463	0.553	0.609	0.991	1.261	0.619	0.863	0.733	0.786	0.765
		(0.058)	(0.017)	(0.041)	(0.321)	(0.528)	(0.001)	(0.183)	(0.077)	(0.083)	(0.055)
	G_k	0.497	0.559	0.615	1.081	1.334	0.651	0.995	0.849	0.820	0.784
		(0.065)	(0.016)	(0.038)	(0.334)	(0.449)	(0.001)	(0.215)	(0.115)	(0.066)	(0.053)
P-P-P	I_1	(0.076)	(0.041)	(0.096)	(0.322)	(0.536)	(0.002)	(0.144)	(0.029)	(0.089)	(0.130)
	H_2	(0.012)	(0.028)	(0.078)	(0.144)	(0.377)	(0.026)	(0.018)	(0.010)	(0.244)	(0.069)
	G_2	(0.072)	(0.042)	(0.097)	(0.309)	(0.532)	(0.003)	(0.139)	(0.026)	(0.094)	(0.128)

amino group in the D-amino acid oxidase-alanine (ES) complex[43] All methods in *Tables* 3 and 4 consistently predict a slightly favored attack at C_4.

Nucleophiles such as carbanions are prediction to attack N_5 more favorably than C_{4a} or C_{10a}, based on data in *Table* 3. This predication is generally borne out experimentally, and is consistent with the electron-deficient nature of N_5 in the total electron density map.[20] The nucleophilic reactivity at C_{4a} is of particular interest, as Brown and Hamilton[44] propose position 4a as the catalytic site of flavoenzyme oxidations.

Admittedly, a ground state reactivity index cannot be used unerringly in discussing the photoreactivity of flavins. However, in the absence of any well established MO photoreactivity indices,[45] perhaps the closest approximation to the photoreactivity index would be the superde localizabilities calculated from Fukui's perturbation treatment[9,26] of the transition state complex, an excited configuration. An attempt to predict the site of photochemical hydrogen abstraction on the riboflavin molecule was made.[3,20] Also the probable photolytic site predicted for benzyl carbanion attack is at N_5 rather than N_1 because of the significantly higher SDN and FOD of the former position. This prediction is consistent with recent observations.[46] This reactivity difference becomes even more pronounced if δ $(N_{10})=0.5$ is adopted in the calculation. The increase in reactivity difference indicates a possible contribution of the N-alkyl side-chain to the reactivity of flavins.[3]

Table 5 shows SDR indices at the methyl

Table 4. SDR and FRD (parenthesized) at selected positions of the flavin nucleus.

Method	atom	N_1	C_2	C_4	C_{4a}	N_5	C_{5a}	C_6	C_9	C_{9a}	C_{10a}
HMO	I_h	1.250	0.505	0.544	1.046	1.259	0.783	1.021	0.944	0.888	0.802
		(0.360)	(0.020)	(0.032)	(0.216)	(0.300)	(0.086)	(0.093)	(0.040)	(0.135)	(0.061)
	H_h	1.242	0.506	0.546	1.058	1.286	0.771	1.037	0.957	0.878	0.808
		(0.373)	(0.020)	(0.031)	(0.216)	(0.295)	(0.080)	(0.096)	(0.053)	(0.123)	(0.060)
	G_h	1.232	0.507	0.550	1.078	1.327	0.756	1.057	0.972	0.866	0.817
		(0.401)	(0.020)	(0.031)	(0.218)	(0.288)	(0.073)	(0.099)	(0.075)	(0.108)	(0.059)
ω=0.5	I_h	1.662	0.497	0.523	1.066	1.174	0.818	1.018	0.996	0.857	0.757
		(0.425)	(0.019)	(0.029)	(0.207)	(0.287)	(0,066)	(0.090)	(0.060)	(0.093)	(0.044)
	H_h	1.645	0.497	0.524	1.071	1.187	0.811	1.030	1.007	0.851	0.760
		(0.426)	(0.018)	(0.029)	(0.208)	(0.281)	(0.063)	(0.094)	(0.066)	(0.087)	(0.044)
	G_h	1.598	0.496	0.526	1.081	1.221	0.790	1.035	1.012	0.833	0.767
		(0.445)	(0.018)	(0.028)	(0.210)	(0.273)	(0.059)	(0.100)	(0.087)	(0,072)	(0.044)
ω=1.4	I_h	4.283	0.510	0.526	1.319	1.077	1.109	1.026	1.281	0.845	0.743
		(0.447)	(0.013)	(0.025)	(0.211)	(0.267)	(0.051)	(0.095)	(0.081)	(0.053)	(0.031)
	H_h	4.435	0.513	0.528	1.352	1.075	1.141	1.065	1.312	0.863	0.743
		(0.441)	(0.013)	(0.024)	(0.210)	(0.264)	(0.051)	(0.096)	(0.078)	(0.054)	(0.030)
	G_h	3.566	0.498	0.520	1.237	1.103	1.003	1.011	1.225	0.798	0.749
		(0.451)	(0.012)	(0.023)	(0.213)	(0.250)	(0.049)	(0.108)	(0.103)	(0.040)	(0.031)
P-P-P	I_2	(0.286)	(0.021)	(0.049)	(0.233)	(0.273)	(0.109)	(0.079)	(0.020)	(0.151)	(0.065)
	H_2	(0.082)	(0.022)	(0.049)	(0.234)	(0.252)	(0.044)	(0.228)	(0.077)	(0.162)	(0.075)
	G_2	(0.242)	(0.022)	(0.050)	(0.226)	(0.267)	(0.108)	(0.103)	(0.014)	(0.157)	(0.065)

Table 5. SDR and FRD at methyl carbons (CH_3)

Isoalloxazine	Methyl Position	SDR*			FDR*			
		HMO	ω=0.5	ω=1.4	HMO	ω=0.5	ω=1.4	P-P-P**
7,8-dimethyl	7	0.334	0.334	0.334	0.001	0.000	0.000	0.010
	8	0.334	0.334	0.334	0.000	0.000	0.000	0.007
7,9-dimethyl	7	0.332	0.332	0.332	0.001	0.000	0.000	0.094
	9	0.332	0.332	0.332	0.001	0.000	0.000	0 004
8,9-dimethyl	8	0.332	0.332	0.331	0.000	0.000	0.000	0.009
	9	0.332	0.332	0.332	0.000	0.000	0.000	0.002
6,7,8-trimethyl	6	0.332	0.332	0.331	0.000	0.000	0.000	0.002
	7	0.332	0.332	0.332	0.001	0.000	0.000	0.009
	8	0.332	0.331	0.331	0.000	0.000	0.000	0.004
6-methyl	6	0.332	0.331	0.331	0.000	0.000	0.000	0.000
7-methyl	7	0.332	0.332	0.332	0.001	0.000	0.000	0.009
8-methyl	8	0.332	0.331	0.332	0.000	0.000	0.000	0.006
9-methyl	9	0.332	0.332	0.332	0.001	0.000	0.000	0.006

*Based on the G_h-model.
**Based on the G_2-model.

carbons. Only the P-P-P method seems to suggest reaciivity difference, generally 8-methyl being more reactive. However, the 8-methyl hydrohen in the group orbitals showed a higher SDR(0.381) than the 7-methyl hydrogen(0.377) by the HMO calculation. FRD values are, on the other hand, reversed (*i.e.*, 0.008 (HMO) and 0.024 (P-P-P) at the 7-methyl hydrogen, 0.007 (HMO) and 0.014 (P-P-P) at the 8-methyl hydrogen). Thus, it appears that restricted or unestricted Hartree-Fock calculations of open-shell flavins be useful in determining the inequivalence of the two methl groups. Indeed, *G*-model caclulations by restricted and unrestricted Hartree-Fock SCF methods indicate distinctively spin densities, not only between the 7- and 8-methylcarbons, but also between C_7 and C_8, and between the 7-and 8-methyl hydrogens of the group orbitals.[47] Even the simplest calculation of the HMO type shows a nonequivalence of the 7-and 8-methyl groups of 7,8-dimethylisoalloxazine semiquinone. Thus, the FRD values are 0.1845 and 0.1409 for C_7 and C_8, respectively, 0.004 and 0.0143 for 7-CH_3 and 8-CH_3, respectively, 0.002 and 0.0105 for the 7- and 8-group orbital hydrogens, respectively.

6. **Singlet $\pi\rightarrow\pi^*$ Transition Energies.** Our previous paper[3] summarizes the results from a detailed study of the singlet transition energies of flavin derivatives. Two features of the work in Ref. 48 are noteworthy. First, the H_2-model fails to predict the lowest transition energh satisfactorily. The results of the H_1model calculations were even worse. Undoubtedly, such a failure on the of the H-model is closely associated with the overestmation of"hyperconjgation" in the pseudo-heteroatom approximation of 7, 8-methyl groups. we have shown a similar inadequacy of the H-model in calculating singlet-triplet transition energies[30] and singlet transition moments[48] for flavins. It is, therefore, necessary to reexamine the pseudo-heteroatom approximat ion with semiempirical integrals other than those published in the literature and used in this work. we have tested the set of methyl integrals obtained by Roos[49] for flavins with similarly unsatisfactory results. Secondly, the I and G mdels resemble each other. This was the case with respect to excited and ground state properties, as discussed earlier.

In conclusion, several MO metheods yield qualitatvely consistent results for flavins and possibly other biomolecules. However, the pseudo-heteroatom (H) approximation to methyl groups is inadequate, and crude MO methods are practically insensitive to differences in methyl treatments. For small molecules, the *H*- model may be satisfactory.[49,50] The HMO method does yield ground state properties in good agreement with the resuits from the P-P-P SCF MO method, provided a proper set of Coulomb and resonance integrals, for example the pullman set,[22,51] is adopted. The spectral quantities are most satisfactorily computed with the P-P-P MO with some modifications, although HMO and ω-SCF HMO may be used for relative spectral correlation wity limited success. Various aspects of the effects of the methyl substitution on electronic structure, photochemial, spectral, and biological properties of flavins have been discussed, in qualitaive fashion. It is suggested that the inclusion of methyl groups in quantum

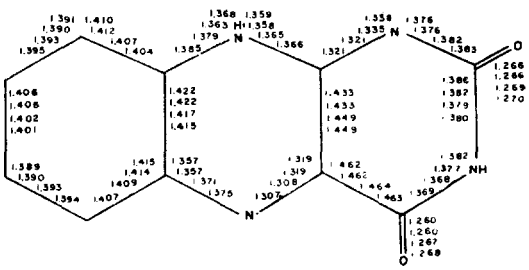

Fig. 9. Bond distances in isoalloxazine calculated by different methods: From top to bottom; HMO, $\omega=0.5$; P-P-P (I_r), and P-P-P (I_a).

biochemical calculations is necessary and warrants further study.

Appendix

Fig 9 shows the bond distances of isoalloxazine calculated by different methods. Data in *Fig.* 9 may be used to compare with the bond distances listed in *Table* 1. *Figs.* 10, 11, and 12 show mobile orders for 7, 8-dimethyl-isoalloxazine, from which the data in *Table* 1 were extracted, and used in the Results and Discussion section of the present paper.

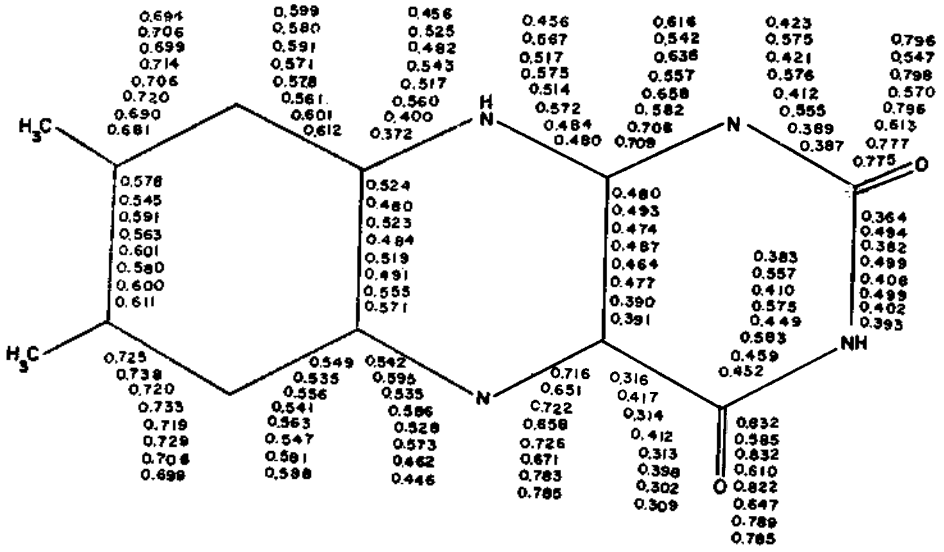

Fig. 10. Mopile bond orders (P_{rs}) of 7, 8-dimethlisoalloxazine (I-model) calculated by different methods: From top to bottom; HMO (H_h), HMO (H'_h), ω=0.5 (I_h), ω=0.5 (I'_h), ω=1.4 (I_h), ω=1.4 (I'_h), P-P-P (I_1), and P-P-P (I_1).

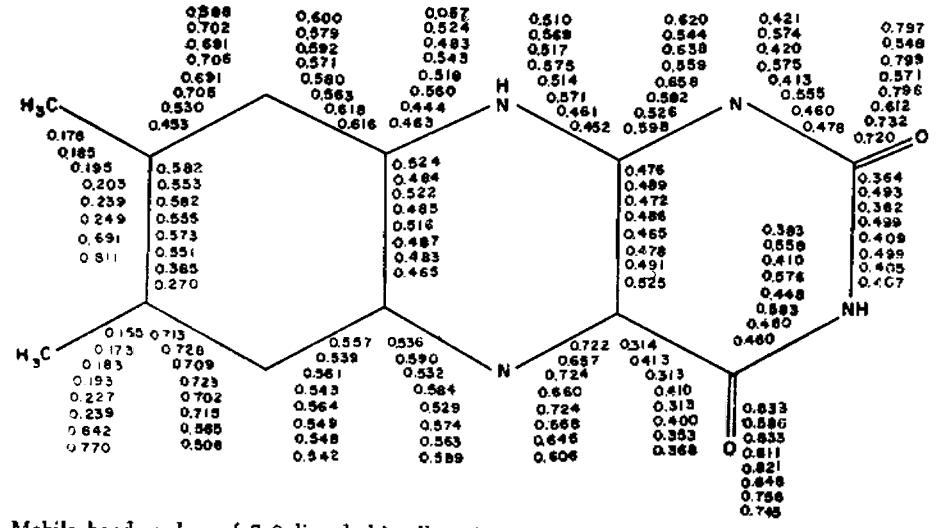

Fig. 11. Mobile bond orders of 7, 8-dimethyl-isoalloxazine calculated by different methods: From top to bottom: HMO (H_h), HMO (H'_h), ω=0.5 (H_h), ω=0.5 (H'_h) ω=1.4 (H'_h), P-P-P (HI), and P-P-P (H_1) (H_2).

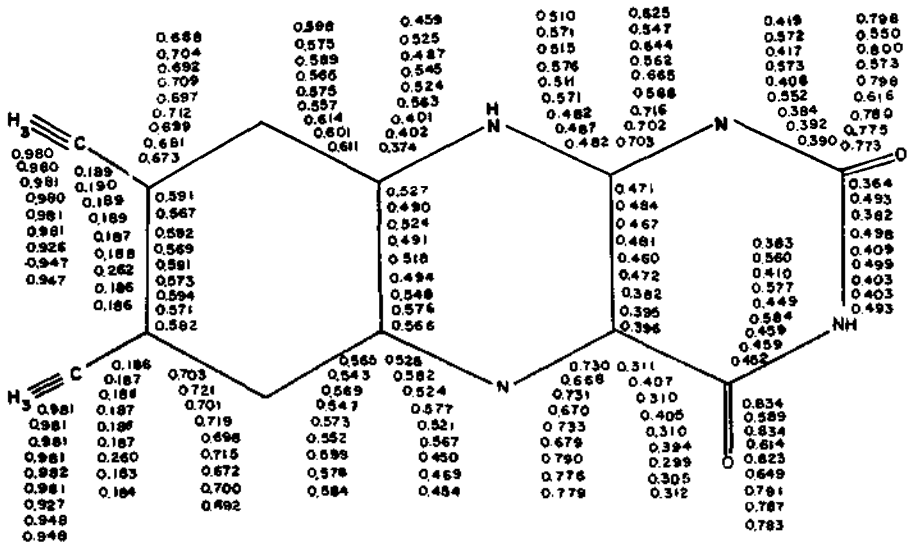

Fig. 12. Mobile bond orders of 7,8-dimethyl-isoal loxazine calculated by different methods: From top to bottom: HMO (G_h), HMO (G'_h), ω=0.5 (G_h), ω=0.5 (G'_h), ω=1.4 (G'_h), ω=1.4 (G'_h), P-P-P (G_1), P-P-P (G_2), and P-P-P (G_3).

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