He(I) and He(II) Photoelectron Spectra of Alloxazines and Isoalloxazines

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Abstract: The He(I) and He(II) photoelectron spectra of isoalloxazines, both in their oxidized and reduced forms, of 1,3-dimethylalloxazine, and of a 5-deazaisoalloxazine derivative were measured. The spectra are interpreted using various methyl-substituted isoalloxazines and by comparison with the results obtained from CNDO/S calculations and photoionization cross sections derived therefrom. The dependence of the electronic properties of isoalloxazines on the redox state and the degree of substitution is analyzed. A critical review of the data obtained from semiempirical MO calculations by various authors and the CNDO/S results show that a few methods give a fairly good prediction of the π-orbital energies only. Without exception the calculated σ-orbital energies contain considerable error. Particularly, all theoretical methods fail to predict a threefold degeneracy in the isoalloxazine orbital level scheme at ~9.6 eV in which both π and σ orbitals are involved. Possible reasons for the failures are discussed. Analysis of the experimental and theoretical results reveals a planar molecular conformation to be the most probable one for an isolated reduced isoalloxazine molecule in the vapor phase, contrary to the bent conformation which was established in solution and in the solid state. Bending therefore is most likely caused by interaction of the molecule with its environment leading to changes in orbital energies. Such interactions may in part be responsible for the ability of the protein-bound flavocoenzyme to be involved in a broad diversity of biological reactions.

Introduction

The electronic structure of isoalloxazines and alloxazines has been the subject of extensive theoretical study and quantum-mechanical calculations. Such research efforts are motivated by the fact that 7,8-dimethyl-10-ribitylisoalloxazine derivatives, commonly referred to as flavins, form the prosthetic group of flavoproteins and participate in reduction-oxidation reactions and electron transport in living organisms. A brief summary of the relevant literature was given in previous work.[2a] The main reason to start comparative studies on alloxazines and isoalloxazines is that the former compounds are decomposition products of riboflavin and shows a very interesting ground and electronically excited state tautomerism, which relates them to isoalloxazines.[2b,3]

Quantum-mechanical calculations, irrespective of their degree of approximation, need rigorous experimental support, especially in the case of molecules like (iso)alloxazines. This was recognized to a greater or less extent in the various publications which appeared in the last 2 decades describing the results from Hückel,[4,5] extended Hückel,[6,7] SCF-PPP,[3,8-15] CNDO/2,[7,16] and MINDO/3[17] calculations. The experimental data used thus far to support the theoretical work were (1) molecular structure and geometry,[6,8,9,13] (2) excited-state energies and optical spectra,[3,8-15,17] (3) correlation with ESR and NMR spectra,[7,8,16,17] and (4) chemical properties derived from the application of Fukui's frontier molecular orbital theory[18] by some authors.[17,19] All these experimentally observable properties used to test the theoretical models, however, generally depend in a very complex way on the orbital structure. For this reason correlation between theory and the experiments mentioned before gives only a rough indication of the reliability of the theoretical method under consideration. Especially when electronic transitions are calculated, incorporation of configuration interaction into the computation is needed to obtain results which reasonably agree with the experiment. This has already been recognized,[8,10-12,14] but the methods employed have the disadvantage that a limited number of singly and doubly excited configurations of only $\pi-\pi^*$ character were allowed to interact. Moreover, these configurations arise from an orbital level scheme which is

without experimental verification. Fox partially solved this problem by performing a direct calculation on the first excited singlet and triplet states of an isoalloxazine derivative in its oxidized form,[17] but this method also left some of the essential features of the electronic structure unverified.

Song[7] has expressed the need to obtain more information about the electronic structure of isoalloxazines by measurements of the ionization potentials. Our discovery that (iso)-alloxazines can be sublimed under appropriate conditions which makes them accessible to vapor-phase emission and absorption spectrometry[20] prompted us to measure also He(I) and He(II) photoelectron spectra. Such spectra, which were not available up till now, allow for a verification of the orbital energies by experiment.

It was already noted by Visser and Müller[21] that CNDO/S calculations were never applied to these molecules, although they can be very useful for the experimentalist. It is now firmly established that the CNDO/S method gives a reliable interpretation and assignment of the bands in ultraviolet photoelectron spectra,[22-26] using Koopmans' theorem.[27] The experiment therefore enables us to test the CNDO/S and the other theoretical methods by new experimental data.

Experimental Section

The compounds studied here were synthesized according to the methods published elsewhere.[2a,20,21] The compound 3,7,8,10-tetramethyl-5-deazaisoalloxazine was a generous gift of Professor Dr. P. Hemmerich, University of Konstanz, West Germany.

Determination of the sublimation temperatures of the compounds under investigation was done by means of a Mettler thermoanalyzer Type 1. Analysis was done under conditions as closely comparable as possible to those which exist in the target chamber of the photoelectron spectrometer. The spectra were measured rather easily on a Perkin-Elmer PS 18 photoelectron spectrometer modified with a Helectros He(I)-He(II) source and calibrated using Xe and Ar lines as an internal calibrant.

The He(I) and He(II) photoelectron spectra of (iso)alloxazines can be measured rather easily on a PS-18 photoelectron spectrometer at temperatures about 40-60 K above the approximate sublimation temperatures as determined by thermoanalysis. In spite of the low count rate (200-800 counts/s) higher temperatures should be avoided because of decomposition of the samples and condensation of (iso)-
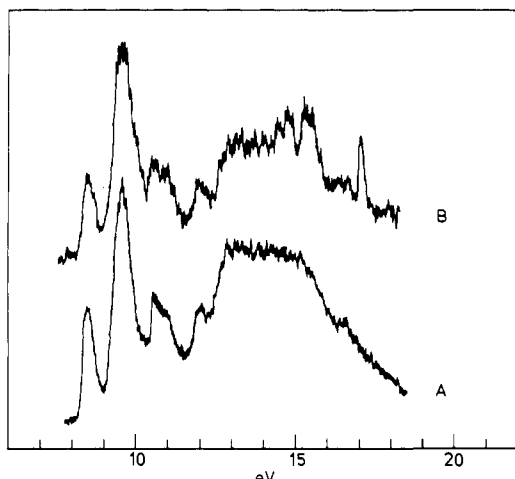
Figure 1. He(I) (A) and He(II) (B) photoelectron spectra of 3,10-dimethylisoalloxazine (1) at 508 K.

Scheme I, Structures of (Iso)alloxazines Investigated by Photoelectron Spectroscopy

Isoalloxazine derivatives:

1: $R_6 = R_7 = R_8 = R_9 = H$
2: $R_6 = R_9 = H; R_7 = R_8 = CH_3$
3: $R_7 = R_8 = R_9 = H; R_6 = CH_3$
4: $R_6 = R_7 = R_8 = H; R_9 = CH_3$
5: $R_6 = R_8 = R_9 = H; R_7 = F$

alloxazine vapor on vital parts in the interior of the spectrometer, causing loss of sensitivity and bad reproducibility in the experiments.

Calculation Procedures

The MO calculations were performed according to the CNDO/S method of Del Bene and Jaffé.[28] The parametrization, however, was taken from more recent work by Kuehnlenz and Jaffé[29] in which mainly the one-center two-electron integrals γ_{AA} were adjusted to obtain better agreement between the calculated and observed spectral properties of a larger variety of molecules than was calculated in the earlier CNDO/S work. The two-center two-electron integrals γ_{AB} were approximated utilizing the Nishimoto-Mataga formula,[30] originally introduced into the CNDO/S framework by Ellis et al.[31]

The spectrum of eigenvalues was shifted by means of the expression derived by Bigelow[22] to fit the calculated eigenvalues of benzene in the CNDO/S2 parametrization of Lipari and Duke[32] to the experimental photoelectron spectrum:

$$\epsilon_i^{cor} = 11.364 \ln (\epsilon_i^{calcd}/4.400) \qquad (1)$$

Although this purely empirical expression was intended for the interpretation of benzene only, it appears to be able to give a reasonable agreement between CNDO/S eigenvalues and experimentally observed ionization potentials even in molecules other than benzene.[24]

Photoionization cross sections were calculated by means of Ellison's method[33] modified by Beerlage and Feil.[34] The modification consists of adjustment of the photoelectron wavenumber k to account for the electrostatic interaction between the free electron and the remaining molecular cation according to

$$k' = (k^2 + \beta)^{1/2} \qquad (2)$$

in which

$$\hbar^2 k^2/2m = \hbar\omega - IP_n \qquad (3)$$

where $\hbar\omega$ represents the incident photon energy, IP_n the ionization potential for the nth MO, and m the electron mass.

Structural data for isoalloxazines were taken from the crystallographic work performed on both the oxidized[13,35-37] and the reduced forms.[36,38,39] Since no structural data are known for alloxazines and 5-deazaisoalloxazine derivatives, the geometry of these compounds was assumed to be the same as that of the oxidized isoalloxazines.
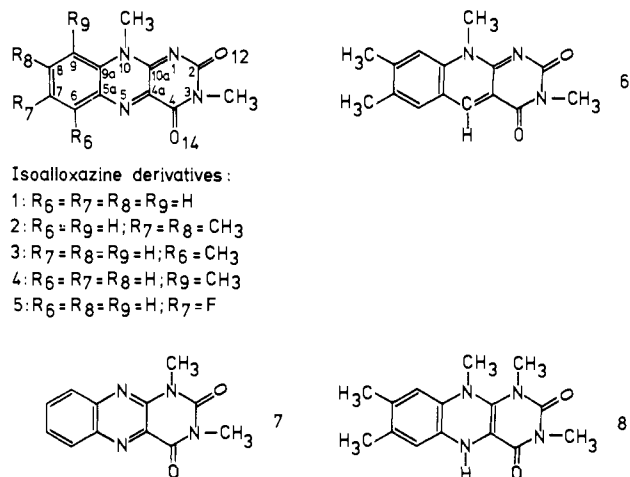
Experimental Results and Spectral Assignments

A, Isoalloxazine Derivatives in the Oxidized Form, The oxidized isoalloxazine derivatives (compounds 1-5; cf. Scheme I) show very similar photoelectron spectra. The full He(I) and He(II) spectra of compound 1 are presented in Figure 1 as representative examples. None of these compounds shows any considerable difference between its He(I) and He(II) spectrum. This indicates large orbital delocalization and it rules out the possibility of using He(I)-He(II) intensity differences as an assignment criterion in these molecules. Large molecules like isoalloxazines evidently do not show any vibrational structure in their photoelectron spectra, so for assignment of spectral bands we are forced to rely on substituent effects and comparison with theoretical data (Figure 2).

At first glance the corrected[22] CNDO/S eigenvalues agree surprisingly well with the observed spectra (cf. Figure 2). However, intensity considerations[40] indicate that it is highly improbable that the intense band, observed between 9 and 10 eV in all spectra, can be assigned to a single π orbital, although this is suggested by the calculation. If this were true, the photoionization cross section of the two highest occupied π orbitals would differ by a factor of 3.5 as deduced from the relative band areas.

For verification a modified plane wave cross-section calculation[34] was performed, using the corrected eigenvalues and eigenvectors of the "ideal" all-proton isoalloxazine model as defined in the next section. The dependence of the cross sections on the β parameter (eq 2) is shown in Figure 3 (0.0 < β < 4.0). Although the first ionization potential (~8 eV) is reasonably far off-threshold, a rather large β value is required to obtain theoretical results which do not imply appreciable intensity differences between the He(I) and He(II) spectra. However, full agreement with the experimentally observed differences cannot be achieved by calculation. On the other hand, there is no β value giving a cross-section ratio of 3.5 for the highest occupied orbitals π_8 and π_9. So, according to the tentative rule by Schweig and Thiel[40] (in first approximation the intensity of a photoelectron band is proportional to the orbital degeneracy), the intense band in the photoelectron spectra of oxidized isoalloxazines should be due to a threefold degeneracy in the orbital level scheme at ~9.5 eV.

This feature was further explored by a study of substituent effects. It is known that π orbitals are destabilized upon methylation whereas introduction of a fluoro substituent stabilizes σ orbitals.[41] Additionally, methyl groups hardly affect σ orbitals and fluoro substituents hardly affect π orbitals. Therefore it should be possible to lift the above-mentioned degeneracy by introducing substituents in appropriate positions
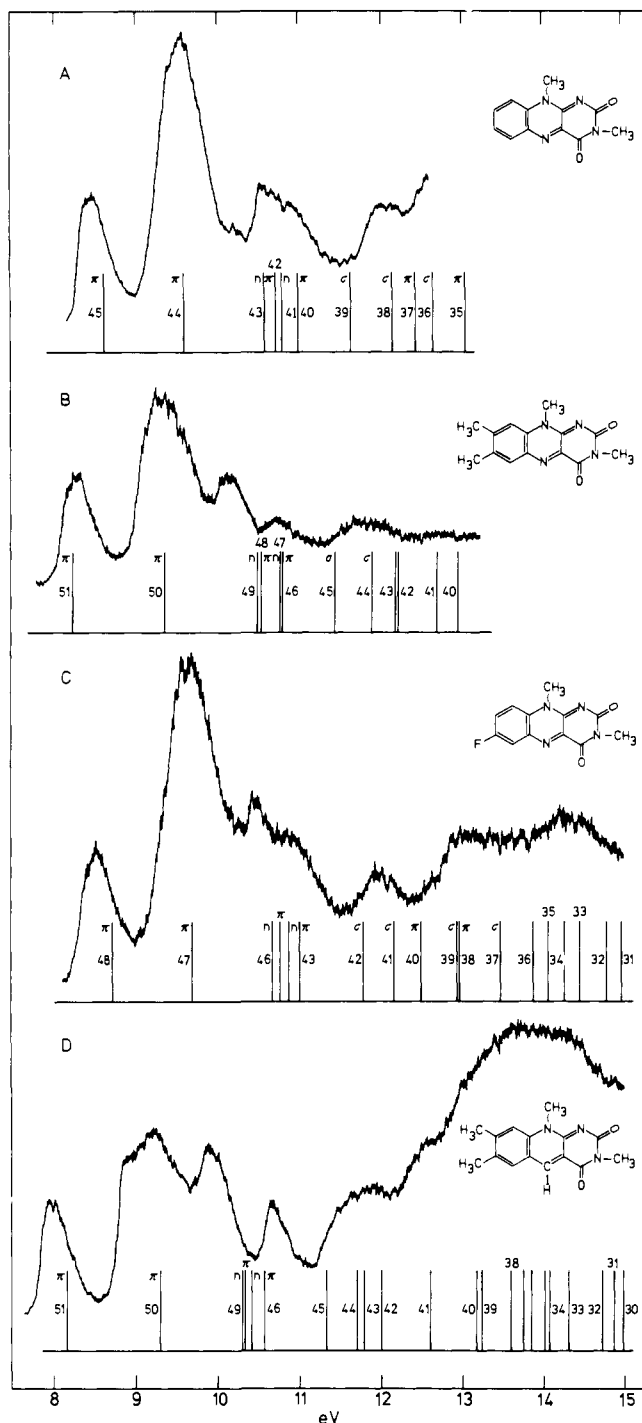
Figure 2. He(I) spectra and corrected[22] CNDO/S eigenvalues (cf. eq 1) of some oxidized isoalloxazine derivatives: A, 3,10-dimethylisoalloxazine (1) at 542 K; B, 3,7,8,10-tetramethylisoalloxazine (2, 3-methyllumiflavin) at 529 K; C, 3,10-dimethyl-7-fluoroisoalloxazine (5) at 490 K; D, 3,7,8,10-tetramethyl-5-deazaisoalloxazine (6) at 513 K. The orbital numbers are counted up from the lowest occupied CNDO/S orbital.

Figure 3. The relation between the photoionization cross section of some orbitals of "ideal[35] isoalloxazine" and the β parameter used to adjust the photoelectron wavenumber in the modified plane wave approximation[34] (cf. eq 2). The isoalloxazine nucleus was assumed to carry only hydrogen atoms as substituents. The orbital energies are given in Table I. The value of $(d\sigma/d\Omega)$ is expressed in arbitrary units.

in the molecule. In this respect the CNDO/S calculations may offer guidance in what particular position a substituent should be placed to obtain an experimentally observable effect.

Inspection of the CNDO/S results frequently shows quasi-degeneracy in the orbital level scheme ($\Delta E < 0.02$ eV). Whenever this occurs, the orbitals involved therein are of *different type* (π *and* σ) and localized in different parts of the molecule. Examination of the two highest occupied orbitals (π_9 and π_8 in the all-proton model) shows them to be localized to a great extent in the benzene subnucleus of the molecule.

The squares of the coefficients in the LCAO expansion corresponding to the centers in the benzene ring add up to 0.56 in π_9 and to 0.85 in π_8. Comparison with benzene itself[42] shows a striking resemblance of π_8 and π_9 with the benzene E_{1g} orbitals. The nodal plane of π_8 intersects the C_7-C_8 and C_{5a}-C_{9a} bonds, whereas the nodal plane of π_9 passes almost exactly through the centers C_6 and C_9. This is in agreement with the pronounced methyl-group effect on the highest occupied orbital as deduced from the first ionization potentials of compounds 1 and 2 (Figure 2 and Table I). Because of the high density on C_6 and C_9 calculated for π_8 ($C \simeq 0.6$) we do expect a similar methyl-group effect on this orbital upon methylation of these centers. For this reason we also studied compounds 3 and 4, although they were found to be thermally less stable than the other compounds, both by thermoanalysis and in the photoelectron spectrometer. Photoelectron spectra of these compounds, however, proved to be measurable without serious interference from decomposition, as long as the temperature was not raised more than 20 K above the sublimation temperature (cf. caption to Figure 4). The low-energy parts of their He(I) spectra are given in Figure 4, showing considerable broadening of the intense second band in the spectrum of compound 3 (Figure 4B) and a distinct shoulder in the corresponding band of compound 4 (Figure 4A). This clearly points toward degeneracy.

For reasons given before it is most likely that degeneracy occurs between σ and π orbitals. Inspection of the two highest occupied orbitals of σ character (σ_{30} and σ_{29} in the all-proton model) shows them to be linear combinations of oxygen and imine-like nitrogen lone pairs. This implicates large through-bond interactions.[43,44] The σ orbitals involved herein are localized in the pyrimidine subnucleus of the molecule. This is supported to some extent by the absence of a fluoro substituent effect upon introducing this atom at position C_7 in the benzene subnucleus (Figure 2C). In order to investigate this prediction more thoroughly the photoelectron spectra of a 5-deazaisoalloxazine derivative were measured (Figure 2D). Formal replacement of N_5 by a C-H fragment causes the intense second

Table I. Experimental and Calculated[a] Ionization Potentials of Various Alloxazines and Isoalloxazines

"Ideal Isoalloxazine"[b]

No.	Calc.	Ass.
39	8.66	π_9
	9.81	π_8
	10.89	σ_{30}
	10.92	π_7
35	11.00	σ_{29}
	11.43	π_6
	11.88	σ_{28}
	12.47	σ_{27}
	12.77	π_5
30	12.83	σ_{26}
	13.47	π_4
	13.57	σ_{25}
	14.24	π_3
	14.33	σ_{24}
25	14.62	σ_{23}
	15.19	σ_{22}
	15.27	π_2
	15.28	σ_{21}
	15.72	σ_{20}
20	15.94	σ_{19}
	16.30	π_1
	16.51	σ_{18}
	16.91	σ_{17}
	17.70	σ_{16}
15	18.75	σ_{15}
	19.15	σ_{14}
	19.27	σ_{13}
	20.34	σ_{12}
	20.85	o_{11}
10	21.38	σ_{10}
	22.47	σ_9

1				2				3				4			
Exp.[c]	No.[d]	Calc.[e]	Ass.[f]	Exp.	No.	Calc.	Ass.	Exp.	No.	Calc.	Ass.	Exp.	No.	Calc.	Ass.
8.47	45	8.62	π	8.22	51	8.21	τ	8.16	48	8.53	π	8.30	g)		π
	44	9.58	π		50	9.33	π	8.97	47	9.20	π	9.09			π
9.54	43	10.58	n	9.33	49	10.47	n	9.29	46	10.54	n	9.48			n
	41	10.79	n		47	10.76	n		44	10.82	n				n
10.55	42	10.71	π	10.09	48	10.51	π	10.23	45	10.70	π				
10.88	40	11.00	π	10.87	46	10.77	π	10.62	43	10.97	π				
11.98	39	11.63	σ	11.84	45	11.42	σ		42	11.43	σ				
	38	12.14	σ		44	11.87	σ		41	11.97	σ				
12.74					43	12.17	σ		40	12.32	π				
	37	12.42	π		42	12.21	π		39	12.51	σ				
	36	12.64	σ		41	12.67	π		38	12.84	π				
	35	13.03	π		40	12.94	σ		37	13.21	σ				
	34	13.27	σ		39	13.53	σ		36	13.53	-				

5				6				7				8[h]			
Exp.	No.	Calc.	Ass.	Exp.	No.	Calc.	Ass.	Exp.	No.	Calc.	Ass.	Exp.	No.	Calc.	Ass.
8.51	48	8.71	π	7.94	51	8.16	π	8.63	45	8.54	π	7.00	55	6.96	π
	47	9.70	π	9.02	50	9.30	π	9.41	44	8.88	π	8.46	54	8.82	π
9.69	46	10.69	n	9.24	49	10.31	n	9.90	43	10.23	n	8.68	53	8.94	π
	44	10.89	n	9.43	47	10.42	n	10.54				9.53	52	10.35	n
10.57	45	10.78	π	9.87	48	10.33	π	10.95				9.83	51	10.71	π
11.06	43	11.03	π	10.64	46	10.58	π		42	11.13	π[j]	10.61	50	10.89	π
12.06	42	11.81	σ	11.83	45	11.34	-		41	11.16	π[j]	11.37	49	11.24	π
	41	12.18	σ		44	11.71	-		40	11.23	π	11.65	48	11.30	σ
13.16					43	11.80	-		39	11.66	n		47	11.39	σ
	40	12.52	π		42	12.01	-		38	12.03	π		46	11.78	σ
	39	12.98	σ		41	12.61	-	11.51	37	12.24	σ	12.25	45	12.42	π
	38	13.00	π		40	13.17	-	12.39				13.25	44	12.80	σ
	37	13.50	σ		39	13.24	σ	13.17					43	12.98	π

[a] Calculated ionization potentials corrected according to Bigelow[22] (cf. Eq. 1); [b] According to the geometry given by Wang and Fritchie[35]; [c] Experimental value (eV); [d] Number of calculated orbital counted up from the lowest occupied orbital = 1; [e] Calculated value (eV); [f] Assignment; [g] No calculation performed; [h] Planar molecular conformation assumed; [j] Mixed: $\sim$50%, π, $\sim$50% σ.
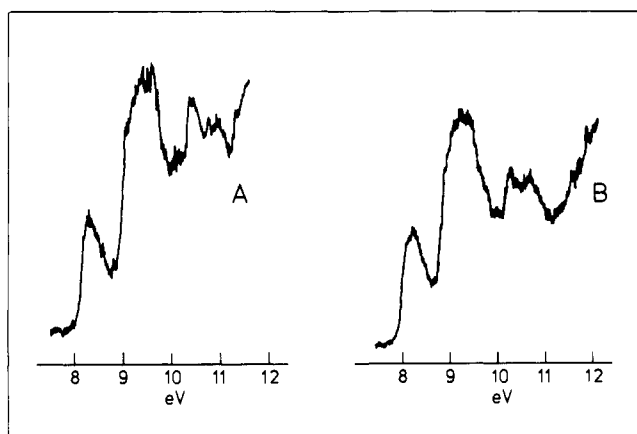

Figure 4. Details of the He(I) photoelectron spectra of 3,9,10-trimethylisoalloxazine (4) at 462 K (A) and of 3,6,10-trimethylisoalloxazine (3) at 497 K (B).

band in the spectrum to split and produces an additional shoulder on its high-energy edge at $\sim$9.5 eV. The remaining spectral characteristics, including the absence of He(I)-He(II) intensity differences, do not differ considerably from those observed in the spectra of compounds 1-5. This result definitely provides strong evidence for a threefold degeneracy in the orbital level scheme of an oxidized isoalloxazine at about 9.5 eV.

Comparison of the CNDO/S results (corrected by Bigelow's formula[22]) with Song's SCF-PPP calculations[10] shows excellent agreement for both the π eigenvalues (cf. Figure 7) and the π eigenvectors.[45] Especially the benzenoid E_{1g} character

of the two highest occupied orbitals is predicted independently by both methods. This supports the spectral assignments. The relevant data are summarized in Table I.
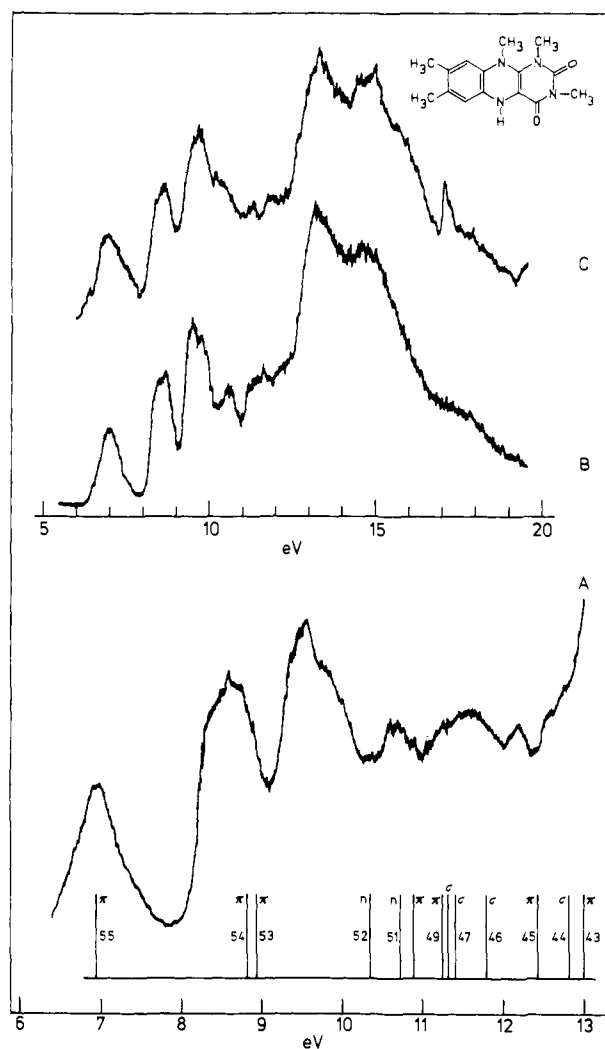
B, 1,3-Dimethylalloxazine. The measurements on alloxazine were confined to the simplest derivative which was found to be stable in the vapor phase. The lack of X-ray crystallographic data on alloxazines makes a theoretical calculation less accurate than for isoalloxazine. This obstructs unambiguous assignment of the spectral bands. Full He(I) and He(II) spectra of 1,3-dimethylalloxazine and an expanded He(I) spectrum combined with the corrected CNDO/S eigenvalues are given in Figure 5. Notwithstanding a large overall difference with the isoalloxazine spectrum, similarities are clearly present. Comparison with the spectra of compound 1 shows the first ionization potential to be only 0.16 eV higher in the case of alloxazine. The intense band observed in the isoalloxazine spectrum is split into two distinct bands in the spectrum of alloxazine. The strongly asymmetric band in the alloxazine spectrum with an origin at 10.54 eV is apparently due to more than one orbital.

The agreement between the calculated and the observed spectrum of alloxazine is not as good as in the case of isoalloxazine. This may be due to an error in the molecular conformation used in the calculation. Because of the lack of X-ray data, the calculations on alloxazine were performed using the molecular geometry of isoalloxazine, except for the position of the N_{10}-methyl group. In alloxazine N_1 was assumed to be methylated instead of N_{10}. The C_{methyl}-N_1 bond distance was taken to be equal to the C_{methyl}-N_{10} bond distance in isoalloxazine and the bond angle toward C_2 was taken as 120°. Both the eigenvalues and eigenvectors are affected considerably by this operation. The benzenoid character and approximate E_{1g}
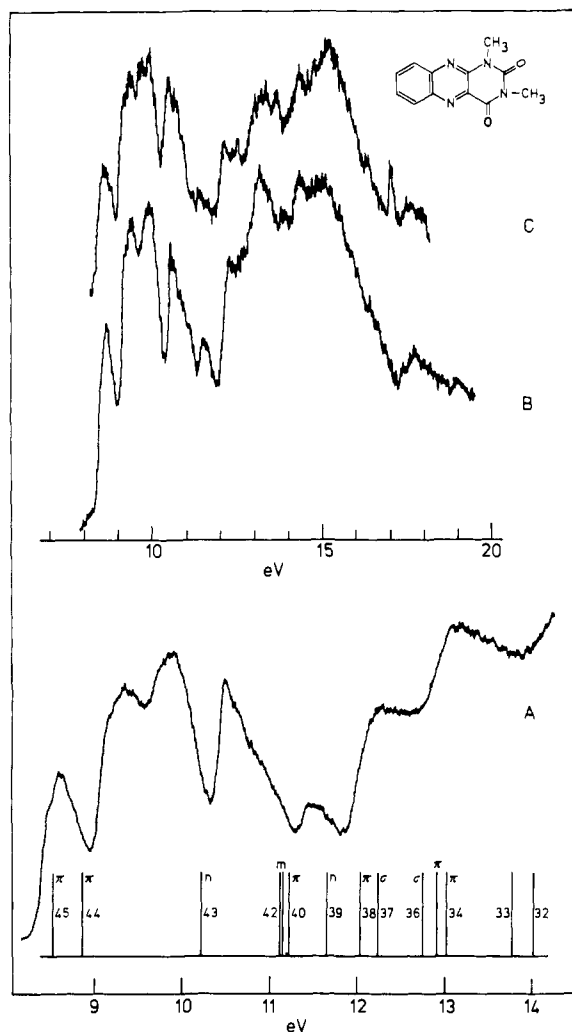
Figure 5. Photoelectron spectra and corrected[22] CNDO/S eigenvalues (cf. eq 1) of 1,3-dimethylalloxazine (7) at 443 K. (A) He(I) spectrum compared with the CNDO/S eigenvalues. The orbital numbers are counted up from the lowest occupied orbital. Orbitals 41 and 42 show considerable σ-π mixing as denoted by "m". (B) Full He(I) spectrum. (C) Full He(II) spectrum.

Figure 6. Photoelectron spectra and corrected[22] CNDO/S eigenvalues (cf. eq 1) of 1,3,7,8,10-pentamethyl-1,5-dihydroisoalloxazine (8) at 451 K. The molecular geometry was assumed to be planar in the calculation. See caption to Figure 5 for further details.

symmetry of the two highest occupied π orbitals as was calculated for isoalloxazine are broken down completely in alloxazine. The highest occupied σ orbital is still of nonbonding character, but now contains mainly the N_5, N_{10}, and O_{14} lone pairs. It also extends more into the benzene subnucleus than in isoalloxazine. The orbitals labeled 41 and 42 in Figure 5 are found to be almost perfect linear combinations of a π and a σ orbital which are both localized mainly on the carbonyl groups and N_3. Orbital 42 contains 41% and orbital 41 contains 54% π character. Calculation of the scalar products between all eigenvectors of compound 1 and those of compound 7 also shows strong mixing of orbitals. Consequently alloxazine has to be regarded as a totally different molecule. This obstructs unequivocal assignment of photoelectron bands. A tentative assignment of a few orbitals is proposed in Table I, based on the following considerations. The orbitals labeled 44 (π) and 43 (n) are both destabilized with respect to the corresponding orbitals calculated for isoalloxazine (compound 1), the destabilization being the largest for the π orbital. This apparently lifts the degeneracy discussed before and yields the order π, π, n for the highest occupied orbitals as is predicted by the calculation. However, quantitatively the agreement between the theory and the experiment is not very good. In the 10.0-12.0-eV energy region a marked He(I)-He(II) intensity dif-

ference is observed, but owing to the strong overlap of spectral bands it is impossible to use this observation as an assignment criterion. Any attempt to draw more conclusions from these data appears to be speculative. More experimental information, particularly crystallographic data, will be necessary for definitive assignment of the alloxazine photoelectron spectrum.

C, 1,3,7,8,10-Pentamethyl-1,5-dihydroisoalloxazine. This compound (cf. Scheme I) was studied as a model for a fully reduced isoalloxazine. Its photoelectron spectra and corrected CNDO/S eigenvalues, *assuming a planar molecular structure,* are given in Figure 6. We made this assumption after an apparent failure of the theoretical method to predict the lower energy part of the spectrum correctly when the structure, as determined by X-ray crystallography,[38,39] was employed. Such a failure was not encountered in the study of the other compounds: when the molecular structure was known, at least the π orbitals were predicted satisfactorily by the theory (cf. below).

The overall features of the photoelectron spectrum of compound 8 are very similar to those of an oxidized isoalloxazine, with one additional band at 6.99 eV, due to the presence of the two extra electrons. The CNDO/S calculation, on the other hand, does not give such a resemblance. Although the coefficients in the highest occupied orbital of compound 8 do not differ considerably from those in the lowest virtual orbital

of the all-proton model, distinct differences are present. The lower occupied orbitals of compound **8** show considerable reorganization compared to the all-proton model. Regarding the distinct shoulder in the photoelectron bands observed at 8.7 and 9.6 eV, it is clear that both bands contain transitions from two different orbitals. This also follows from the band areas relative to that of the band at 6.99 eV and from the application of the tentative rule by Schweig and Thiel[40] (cf. above). The orbitals labeled 54 and 53 in Figure 6 formally originate from the highest occupied orbitals π_9 and π_8 of the all-proton model. Their reorganization, however, causes complete loss of the benzenoid E_{1g} character and a concomitant destabilization of π_8 by 0.87 eV. This is confirmed by the shape of the photoelectron band observed from compound **8** at 8.7 eV. As in the oxidized compounds, the nonbonding orbitals 52 and 51 are calculated to be about 1 eV too low in energy. Their energy difference has increased with respect to the oxidized compounds, which is confirmed by the shape of the band at 9.6 eV. The next band in the photoelectron spectrum likely can be assigned to a π orbital. It is impossible to assign more bands owing to the severe crowding of orbitals below -11 eV in the level scheme and the absence of other assignment criteria.

These results support our preceding conclusion that the intense second band in the spectra of the oxidized derivatives is due to a threefold degeneracy. Reduction lifts this degeneracy and destabilizes the π orbital involved considerably. Ionization potentials and calculated data are collected in Table I.

Calculated Results

The first CNDO/S calculations were performed on an oxidized isoalloxazine skeleton carrying only hydrogen atoms as substituents. Although this is a molecule which does not exist in nature (it would tautomerize to alloxazine), for symmetry reasons it was very useful as a theoretical model. From crystallographic data[13,35-37] it is known that oxidized isoalloxazines have a planar ring system. A planar "all-proton model" is thus a reasonable, simplified model which contains the molecular plane as the only symmetry element. Such a model will at least give unambiguous σ-π separation.

In the methylated derivatives σ-π separability is highly dependent on the rotation of methyl groups. For methylated isoalloxazines in the solid state the angle of rotation of the methyl groups is determined to be such that the molecular plane is not retained as a symmetry element.[37] This may be different in the vapor phase, but there are also C–H bond-length differences within one particular methyl group.[37] Additionally, the all-proton model was required in the calculation of cross sections because of the fact that the modified plane wave computer program was limited to planar molecules.

First the all-proton model was used to test if the small differences in molecular geometry derived from the various crystallographic experiments[13,35-37] affect the results of the CNDO/S calculations. It was established that the eigenvalues did not change by any experimentally observable amount. Moreover, the sets of eigenvectors obtained on different molecular conformations did not show a deviation from orthonormality greater than 1.5% when compared to each other. This was established by the calculation of scalar products between eigenvectors. Consequently these geometry differences are negligible.

Secondly, the influence of substitution and rotation of methyl substituents was investigated. When the coordinates of the hydrogen atoms were not given explicitly in the literature, we used standard bond distances and bond angles[46] in the calculations. Small variations in these also proved to be of no importance. The degree of σ-π mixing was explored primarily by verification to what extent the calculated linear combinations of the methyl group hydrogen 1s functions (denoted by *a, b,* and *c*) satisfy the group orbital approximation:[47]

$$\Phi_1 = 3^{-1/2}(a + b + c)$$

$$\Phi_2 = 2^{-1/2}(b - c)$$

$$\Phi_3 = 6^{-1/2}(2a - b - c) \tag{4}$$

When the conformation is such that the (iso)alloxazine molecular plane contains a proton of each methyl group ("eclipsed" conformation), the molecule has perfect C_s symmetry, like the all-proton model. In this case Φ_1 and Φ_3 have A′ symmetry and Φ_2 has A″ symmetry which makes Φ_2 the only group orbital which can be part of a π orbital. Rotation of methyl groups around the central C–C or C–N bonds over an angle of 90° with respect to the previous situation ("staggered" conformation) breaks down C_s symmetry and, consequently, σ-π separation. Nevertheless, now Φ_3 approximately has the proper symmetry to be part of a π orbital. This behavior is found to a good degree of approximation in the CNDO/S results. Rotation of methyl groups causes formal interchange of Φ_2 and Φ_3 in the eigenvectors whose corresponding eigenvalues remain constant within 1%. This finding supports the group orbital approximation made by Song in his SCF–PPP–CI calculations.[10,45] Whenever σ-π mixing is introduced by rotation of methyl groups it is found to occur primarily on the centers carrying the largest negative charges, i.e., O_{12}; O_{14}; N_1 and N_3.

This analysis was pursued by the calculation of correlations between the CNDO/S eigenvectors. Because of symmetry-determined σ-π separation in the all-proton model, the scalar products between the eigenvectors of this model and those of compounds **1–8** were calculated to examine the character changes of the eigenvectors upon substitution and reduction. By inspection of the CNDO/S eigenvectors it was found that the highest occupied orbitals and the lowest virtual orbitals of (iso)alloxazine do not acquire appreciable density on the methyl or proton substituents. The majority of the eigenvectors can be expressed on an AO basis consisting of the 64 2s and 2p functions corresponding to the 14 ring atoms and the two oxygen atoms in the molecular frame. Scalar products between σ eigenvectors of the all-proton model and vectors derived therefrom by neglecting all hydrogen 1s AO coefficients show 1:1 correlations within an error of 5% for the eigenvectors corresponding to eigenvalues greater than -18.47 eV (-16.30 eV after Bigelow correction). Similar results were obtained with the structure of compound **1**. Therefore, in the calculation of the scalar products between the eigenvectors of two derivatives carrying different substituents in the same ring position (e.g., methyl group vs. proton), the AO contributions of both substituents were simply omitted to obtain contracted eigenvectors on the same AO basis. The scalar product between eigenvector j of molecule "a", denoted by $\langle^a\Psi_j|$, and eigenvector k of molecule "b", denoted by $|^b\Psi_k\rangle$, was thus approximated by

$$\langle^a\Psi_j|^b\Psi_k\rangle = \left[\sum_\mu {}^a c_{\mu j}{}^2\right]^{-1/2}\left[\sum_\nu {}^b c_{\nu k}{}^2\right]^{-1/2}\left[\sum_\lambda {}^a c_{\lambda j}{}^b c_{\lambda k}\right] \tag{5}$$

in which ${}^a c_{\mu j}$ denotes the coefficient of the μth AO in the jth MO of molecule "a". Summation runs over all the retained basic functions.

A. Influence of Substitution. Representative data on the calculated correlations between the orbitals of the all-proton model and those of some oxidized derivatives are given in Figure 7 and Table II. Throughout all calculations a "staggered" conformation was adopted for the methyl groups. Admixture of other orbitals into the two highest occupied orbitals upon substitution is fully negligible. Even the formal replacement of N_5 by a C–H fragment in compound **6** does not have an appreciable influence on these orbitals which, consequently, retain their benzenoid E_{1g} character. At lower orbital

Table II. Correlations between the CNDO/S Eigenvectors of Oxidized Isoalloxazines

Compound	Orbital	39 π_9	38 π_8	37 σ_{30}	36 π_7	35 σ_{29}	34 π_6	33 σ_{28}	32 σ_{27}	31 π_5	30 σ_{26}	29 π_4	28 σ_{25}	Other Orbitals	Main Character
1	HOMO=45	.9979													π
	44		-.9987												π
	43			.9613	-.1037	-.1786	.1482								n
	42			.1719	.8608		-.4679								π
	41			-.1959		-.9787									n
	40			-.1049	.5293		.8215			.1273					π
	39							-.9870	.1150						σ
	38							.1342	.9725				.1130		σ
	37						-.1834			.9451	.2215				π
	36										-.9902				σ
	35						-.1374			.2766		-.8732	.1334	$.1690\ \vert\pi_3\rangle + .2788\ \vert\pi_2\rangle$	π
	34							.2464				-.1592	-.9231	$-.1493\ \vert\sigma_{23}\rangle$	σ
2	HOMO=51	-.9945													π
	50		-.9957												π
	49			-.9485	.1726	.2201									n
	48			-.1711	-.9677						-.1136				π
	47			-.1860		-.9049	-.3781								n
	46			-.2053		-.3790	.8915								π
	45							.9852							σ
	44								.9742					$.1488\ \vert\sigma_{23}\rangle + .1584\ \vert\sigma_7^*\rangle$ $+.1047\ \vert\sigma_{12}^*\rangle$	σ
3	HOMO=48	-.9976													π
	47		-.9955												π
	46			.9544		-.2409								$-.1013\ \vert\sigma_{11}^*\rangle$	n
	45			.1212	.8803		-.4385								π
	44			-.2663		-.9612									n
	43				-.4899		-.8505			-.1083					π
	42							-.9822						$.1074\ \vert\sigma_{10}^*\rangle$	σ
	41							.1494	-.9763					$-.1257\ \vert\sigma_{23}\rangle$	σ
	40						-.1673			.9007	.1001	.3396		$-.1260\ \vert\pi_3\rangle$	π
	39									.1269	-.9759				σ
	38				.1105					.4129		-.8405	.1360	$.1938\ \vert\pi_3\rangle + .1572\ \vert\pi_2\rangle$	π
	37						-.1550					-.1723	-.9318	$-.1535\ \vert\sigma_{23}\rangle - .1120\ \vert\sigma_9^*\rangle$	σ
5	HOMO=48	.9967													π
	47		.9952												π
	46			.9571	.1509	.1438	-.1706								n
	45			.2161	.8995		-.3654								π
	44			.1560		.9818									n
	43			.1288	-.4285		-.8760			-.1173					π
	42							.9600	-.1530		-.2275				σ
	41							.1611	.9697						σ
	40						-.1778			.9562	.1749				π
	39						-.1356			-.6851	-.3769	-.5914		$.1010\ \vert\pi_2\rangle$	σ
	38									-.2167	-.2637	.8222	-.3368	$-.1738\ \vert\pi_3\rangle - .2196\ \vert\pi_2\rangle$	π
6	HOMO=51	-.9946													π
	50		-.9909												π
	49			.9437		.2727	.1052	.1027							n
	48				-.9712	-.1022				.1482					π
	47			-.1631		.8829	-.1237	-.3387	.1394						n
	46			-.1401			.9746								π
	45					-.3184		-.5850	.6397		-.1019		.2849	$.1810\ \vert\sigma_{23}\rangle$	σ
	44							-.5466	-.5003	-.4867	.3252		.1209	$-.1264\ \vert\sigma_{24}\rangle - .1614\ \vert\sigma_{23}\rangle$ $+.1045\ \vert\sigma_{20}\rangle - .1279\ \vert\sigma_6^*\rangle$	σ
	43				-.1462			.3409	.2593	-.8406	-.1785	-.1052			π

Column numbers: Eigenvectors of unsubstituted "ideal[35] isoalloxazines". Row-numbers: Eigenvectors of substituted isoalloxazines (Various compounds; numbers refer to Scheme 1); HOMO = Highest Occupied Molecular Orbital; Numbers are counted up from the lowest occupied CNDO/S orbital.

energies mixing primarily occurs within the π and σ systems separately. Only in the case of almost degenerate π and σ orbitals is mutual admixture found, ranging from 5 (compounds **1** and **3–5**) to 14 (compound **2**) and 21% (compound **6**). Remarkably, compound **3** shows small admixture of the antibonding orbital σ_{11}^* of the all-proton model into its highest nonbonding orbital, labeled 46. The orbital σ_{11}^* is calculated to be localized for 88% on the centers N_1, C_2, N_3, O_{12} and the proton substituent on N_3, about the same molecular region where orbital 46 is localized.

The results obtained on alloxazine (compound **7**) are omitted here for two reasons. First the calculation yields a large number of small scalar products and secondly there is bad agreement of the CNDO/S eigenvalues with the photoelectron spectrum. A correlation diagram cannot be given with these data. Only the two highest occupied alloxazine orbitals can be expressed approximately as linear combinations of π_8 and π_9 of the all-proton model, the minus combination having the highest energy. Both alloxazine orbitals, however, suffer from serious admixture of a large number of other orbitals, both real and virtual. So, also in a theoretical description alloxazine turns out to be totally different from isoalloxazine.

B. Influence of Reduction. Mutual correlations between orbitals of isoalloxazine in its oxidized and reduced forms,
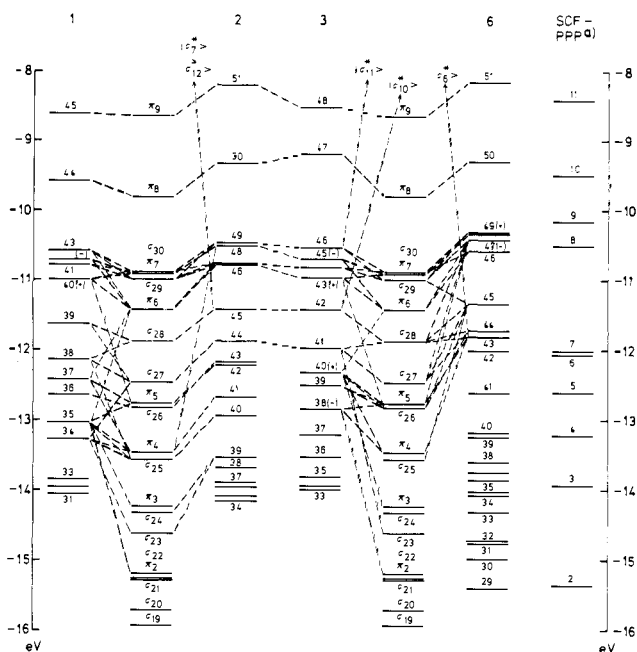
Figure 7. Correlation diagram of the CNDO/S eigenvectors of some substituted isoalloxazines with those of the unsubstituted "ideal isoalloxazine" nucleus.[35] The numbers refer to the compounds given in Scheme I. The scalar products > 0.3 are indicated by heavy dotted lines. The orbital energies (calculated values) are given in Table I; the scalar products are given in Table II; (a) = SCF-PPP eigenvalues obtained by Song.[10,45]

respectively, are of particular interest in connection with the function of the molecule in living organisms, as was denoted before. In first approximation one expects the two additional electrons, taken up by the molecule upon full reduction to a hypothetical divalent anion, to enter into the lowest unoccupied orbital (LUMO) of the oxidized form. Assuming no conformational change, the only consequence for a SCF-LCAO-MO description within this picture is a change of the Fock matrix elements due to an alteration of the density matrix only. Subsequent protonation giving the neutral hydroquinone will be of greater influence because of the addition of two functions to the AO basis set. Both processes will cause reorganization but still a high scalar product between the LUMO of the oxidized form and the HOMO of the reduced form is to be expected.

Severe breakdown of a correlation diagram will likely originate from geometry differences existing between the oxidized and reduced forms of isoalloxazine.[35-39] Contrary to the quinoid form, all 1,5-dihydroisoalloxazines have a nonplanar structure, consisting to a good degree of approximation of two planar parts, bent along the N_5–N_{10} axis.[38,39] In the calculations on compound **8** two geometries were tested. The first one (structure A) was based on a derivative[38] carrying a bromine atom in position 9 and an acetyl group in position 5, whereas in the second one (structure B) the acetyl group was changed into a hydrogen atom.[39] For structure A hydrogen atoms were generated at standard bond distances and bond angles[46] and the bromine atom and acetyl group were replaced by hydrogen atoms at appropriate bond distances without altering the bond angles. In structure B all hydrogen atoms were determined by X-ray crystallography, restricting alteration to replacement of the bromine atom. Nevertheless, both structures give rise to CNDO/S eigenvalues which do not agree with the photoelectron spectrum, agreement being particularly bad in case of structure A. After application of the Bigelow correction the first ionization potentials are found to be too high by 0.94 and 0.61 eV for structures A and B, respectively. This difference can be ascribed to a stereochemical difference of the proton at N_5 in structures A and B. Owing to bending, the pyrazine
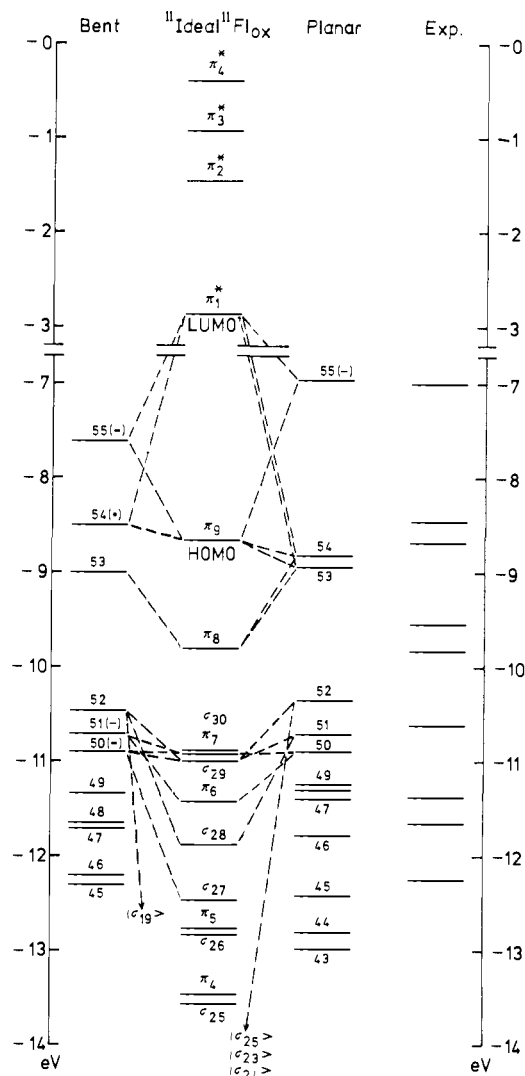

Figure 8. Correlation diagram of the CNDO/S eigenvectors of 1,3,7,8,10-pentamethyl-1,5-dihydroisoalloxazine (**8**) with those of the unsubstituted "ideal isoalloxazine" nucleus,[35] denoted by "Fl_{ox}" to indicate the oxidized form. Comparison is made for the bent and planar structures[39] and the experimental values (exp). Only the scalar products > 0.3 are indicated; the values are given in Table III. The orbital energies (calculated values) are given in Table I.

ring in the molecule acquires the boat conformation with the N_5-acetyl group in an axial position due to steric effects.[38] Formal replacement of this acetyl group by a hydrogen atom causes large overlap between the hydrogen 1s and the N_5 nitrogen $2p_z$ functions leading to a bonding orbital and a concomitant decrease of the orbital energy. This creates a protonated π orbital, however, which is a physically unrealistic situation, as confirmed by the real position of the N_5 proton as determined in structure B.[39] Contrary to the discussion given by Norrestam and von Glehn,[39] direct conversion of their fractional into Cartesian coordinates undoubtedly shows the N_5 proton to be in an equatorial position. The general features of the CNDO/S results therefore agree better with experiment for structure B as compared to structure A. Moreover, an INDO calculation[48] on structure A fails to converge, whereas such a calculation on structure B converges normally. However, only a planar system of three condensed rings, as derived from structure B by adjustment of the (4a–5–5a) and (9a–10–10a) bond angles, has a set of eigenvalues which agree as satisfactorily with the experiment as those of the oxidized compounds.

Consequently, the calculations of scalar products gave

Table III. Correlations between the CNDO/S Eigenvectors of an Oxidized and a Reduced Isoalloxazine

	Orbital	44 * π_5	42 * π_3	41 * π_2	40 * π_1	39 π_9	38 π_8	37 σ_{30}	36 π_7	35 σ_{29}	34 π_6	33 σ_{28}	Other Orbitals	Main Character
	HOMO-55	-.1450	.1843	.1581	-.7577	.3661			.1815		.1533		Dot product with 3 orbitals >.1	π
	54		.1391	.1414	-.4117	-.7277	.1481		-.2687		-.1014		Dot product with 7 orbitals >.1	π
	53					.1624	.8868						Dot product with 5 orbitals >.1	π
	52								-.1751	-.3853		.5997	{.3072 $|\sigma_{19}>$; Dot product with 18 other orbitals ∿.1	n
BENT	51								.4479		.8209		Dot product with 2 orbitals	π
	50							.3559	.2051	-.5067			{-.3077 $|\sigma_{27}>$ -.2223 $|\sigma_{14}>$ +.3058 $|\sigma_4^*>$; Dot product with 12 other orbitals >.1	n
	HOMO-55	.1425	-.1563	-.1525	.8383	-.3770	.1043		-.1100				.1939 $|\pi_5>$	π
	54		.1681	-.3446	-.6574	.5438			-.2743				-.1243 $|\pi_4>$	π
PLANAR	53			.1560	.4858	.8256			.1496					π
	52					.1050	-.1632			.7595	-.1063	-.1981	Dot product with 10 orbitals >.1	n
	51						-.1412			.8910	.1706	-.2999	Dot product with 3 orbitals >.1	n
	50								.4873	.1631	-.8419			π

Row numbers: Eigenvectors of compound 8 in the bent and planar molecular conformations[39]. For further details: see annotations to Table II.

reasonable correlation diagrams for structure B and a planar geometry only. The results are given in Figure 8 and Table III. Considerable reorganization of the orbitals is found to occur upon reduction, even in the case of a planar structure. Presumably reorganization will also be due to differences in bond distances existing between the oxidized and reduced forms of isoalloxazines.[36] The highest three occupied orbitals of the reduced form, however, can be assigned as π orbitals regarding the main character as estimated from the correlation diagram. The HOMO (55) of the reduced form predominantly is a minus linear combination of the HOMO (π_9) and LUMO (π_1^*) of the (oxidized) all-proton model, for both the non-planar (B) and planar structures. The orbitals π_8 and π_9 mix heavily upon reduction to give the orbitals labeled 53 and 54. Especially in case of a planar structure these orbitals differ only by 0.12 eV in energy, giving strong mixture and, consequently, complete breakdown of benzenoid E_{1g} character. Owing to admixture of π_1^*, the orbitals 53 and 54 also acquire higher density outside the benzene subnucleus as compared to the oxidized form. Admixture of other orbitals into 53, 54, and 55 is limited to 29% in case of the bent structure and to 15% for the planar conformation. The redox properties of the molecule therefore are governed mainly by π_8, π_9, and π_1^*. Similar results were obtained in a calculation of the correlation diagram with respect to the eigenvector of compound 2.

Discussion

Comparison of the isoalloxazine photoelectron spectra with the results of the applied theoretical methods[4-17] shows that none of these methods, including the present CNDO/S calculation, is capable of predicting the orbital energies accurately. Reviewing the available data[4-17] we will skirt the extended Hückel method because of its extremely poor agreement with the experiment.[4-7]

The SCF-PPP method predicts the experimental π ionization potentials surprisingly well for both the oxidized and reduced forms of isoalloxazine. There is some dependence on the particular parametrization chosen, because of which Song's results[10,45] agree better with the experiment than those of Grabe,[8] who predicts the π ionization energies systematically too low. Regarding the complexity of the photoelectron spectra caused by (near) degeneracy of π, n, and σ orbitals, however, a π-electron calculation like SCF-PPP cannot serve as a guide to spectral assignment unless at least comparison with all-valence electron calculation is made.

The power of the CNDO/2 method[7,16] to predict photoelectron spectra is extremely poor. For this reason we omitted our own CNDO/2 results, which agree with those of Song[7] as far as the eigenvalues are concerned. The eigenvectors were found to be different, presumably owing to geometry differences (Song had to assume a molecular geometry by the lack of crystallographic data).

Before the application of corrections, the results from CNDO/S and INDO calculations are rather similar, except for random differences in energy up to ±2 eV for resembling eigenvectors. This leads to differences in level ordering between the two methods. Both methods, however, predict ionization potentials which are systematically too high and orbital energy differences which are systematically too large. The Bigelow formula[22,23] partly compensates this error.

The MINDO/3 calculation, which was only applied to oxidized isoalloxazine derivatives,[17] yields π-orbital energies which agree perfectly with the experiment, the SCF-PPP method, and the corrected CNDO/S π orbital energies. Differences are as small as a few tenths of an eV. Conversely, the calculated n and σ orbital energies are systematically too high. The highest n orbital is even predicted to be quasi-degenerate with the highest occupied π orbital. According to the MINDO/3 method, crowding of orbitals in the −8 to −12 eV energy region is that large that the corresponding photoelectron spectrum should be completely structureless. This is in contrast with the experiment.

Thus, only π orbitals are predicted satisfactorily by the SCF-PPP, the (corrected[22]) CNDO/S, and the MINDO/3 methods, the only reservation being our lack of knowledge of the MINDO/3 eigenvectors. Apart from the necessity to correct the CNDO/S eigenvalues Del Bene and Jaffé[28] state correctly that their CNDO/S method includes the simpler π-electron theory as a special case. However, it would be an exaggeration to say that for economic reasons one should confine oneself to π-electron calculations. Firstly, this analysis is restricted to orbital energies exclusively; secondly, awareness of the intrinsic errors of the NDO methods may preclude wrong interpretations.

Even when the Bigelow formula[22] (eq 1) is applied, the CNDO/S calculations on isoalloxazines invariably predict the n-orbital energies about 1 eV too low. Conversely, the correction method[22] adjusts the π-orbital energies satisfactorily. This can be ascribed to the fact that the n orbitals are spatially more localized than the π orbitals, owing to which calculational errors may accumulate in the former orbitals. Apart from the intrinsic errors in any particular SCF calculation, the neglect of the electronic correlation and reorganization cause such errors.[41] The correlation error originates from the multiplication of probability distributions of electrons in any SCF calculation; i.e., the probability of finding two electrons in the

same region of space is given equal statistical weight as finding them largely separated.[49] The major contribution to this error originates from two electrons of different spin occupying the same spatial orbital.[49] Because the electronic repulsion will tend to keep such electrons apart, it is found that the repulsion integrals are generally taken to be too large in an ab initio SCF calculation.[49] In a semiempirical calculation like CNDO/S the parametrization only may compensate this error in an average way,[49] so certain orbitals still may contain residual errors. However, neglect of correlation destabilizes the orbitals owing to an overestimation of the electronic repulsion. Therefore, the error in the isoalloxazine n-orbital calculations must be due to the neglect of reorganization and/or intrinsic shortcomings of the calculation. Obviously, the reorganization will be important because a loalized hole is created by ionizing an electron from the n orbitals. De Bruijn[50] has pointed out that an important intrinsic error of all NDO theories arises from incorrect resonance integrals. The one-center integrals $h_{\mu\mu}$ and the two-center integrals $h_{\mu\nu}$ (μ and ν nearest neighbors) were shown to be too negative, which is partly compensated by an opposite error in $h_{\mu\nu}$ for second neighbors. So the diagonal elements of the core Hamiltonian[51]

$$H_{ii} = \sum_{\mu,\nu} c_{\mu i} c_{\nu i} h_{\mu\nu} \tag{6}$$

are too negative. Because $\sum_{\mu,\nu} c_{\mu i} c_{\nu i}$ is increasingly positive toward lower bonding MOs, the error in H_{ii} increases in that direction, causing a spurious divergence in the orbital level scheme. This explains the success of the correction methods by Bigelow[22,23] which compress the divergence. In case of a deviation from an uniform charge distribution these errors increase appreciably.[50] This situation is encountered in case of isoalloxazine. The calculations predict a large dipole moment due to charge differences on the carbonyl groups and, particularly, in the n orbitals localized thereon. Typical values range from 6.1 (compound 8) to 10.7 D (compound 6).

Probably the threefold degeneracy in the isoalloxazine orbital level scheme in the oxidized state at −9.6 eV is responsible for some of the molecule's peculiar properties such as the pronounced solvent influence on the $S_0 \rightarrow S_2$ electronic transition.[2a,52] Based on its red shift observed on going to polar solvents assignment to an intramolecular charge transfer transition is made,[52] whereas theory predicts a $\pi-\pi^*$ transition at the corresponding wavelength.[10] It should be realized, however, that singly excited configurations, which most likely contribute considerably to the S_2 state in a configuration interaction scheme, will arise from the concerned set of degenerate n and π orbitals. Such configurations may be almost degenerate too, depending on the values of Coulomb and exchange integrals. One of these configurations surely will be a $\pi-\pi^*$ transition carrying large oscillator strength, but owing to the admixture of n-π^* character and intramolecular charge transfer character (owing to the spatial separation of n and π orbitals) a strange composite S_2 state is expected. In this respect strong perturbations like H-bond formation cannot be described properly in a π-electron theoretical framework as was done recently.[14,15]

The small admixture of antibonding character into the highest nonbonding MO of compound 3 (cf. Figure 7 and Table II) provides another example of the considerable influence of small perturbations. In this respect we do not expect the thermal instability of compounds 3 and 4 to be a fortuity, but to originate from electronic properties.

Comparison of the theory (of which the intrinsic errors are known) and experiment shows the reduced isoalloxazine derivative (compound 8) to have a planar structure with sp² hybridization on N_5 and N_{10}. This is at variance with the bent structure found by X-ray crystallography[38,39] and by ¹H NMR coalescence spectroscopy carried out in solution.[48,53] Crys-

tallography shows the presence of two enantiomers (being each other's mirror image) which occur in a 1:1 molar ratio in the solid state.[36,38,39] So, if the bent structure is the most stable one, a plot of the total energy of the molecule vs. the dihedral bending angle gives a double minimum potential energy curve. This curve will be perfectly symmetrical with respect to a dihedral angle of 180° at which a maximum occurs constituting a "transition state" of energy $\Delta U^{\ddagger}$ relative to the minima. Suppose the minima to be localized at dihedral angles of 180° ± α. Typical values[36] range from $\alpha = 36°$ to $\alpha = 9°$ (the latter value refers to a protein-bound isoalloxazine). According to Tauscher et al.[53] $\Delta U^{\ddagger}$ should be about 45 kJ mol⁻¹. Owing to mirror symmetry both enantiomers will have the same orbital energies; hence they will have the same photoelectron spectrum and any change in their thermal equilibrium therefore will be unobservable. So, except for two details, we adopt the interconversion scheme for the enantiomers as proposed by Tauscher et al.[53] where both ring inversion and N inversion in the pyrazine ring occur. First we reject the proposal that only N_5 should undergo inversion. The absence of N_{10} inversion would cause interchange between axial and equatorial positions for the N_{10}-methyl substituent giving appreciably different photoelectron spectra for both enantiomers. If this were the case, the spectral bands would have been broadened considerably and the spectrum would have been temperature dependent, contrary to the actual measurements. The second exception is the high probability of an all-coplanar transition state regarding the agreement between the experiment and the calculation on such a conformation. Consequently, if α and $\Delta U^{\ddagger}$ are of the order of magnitude as quoted before, the population of the transition state is negligible under our experimental conditions (Boltzmann factor of 1.4×10^{-5} at 450 K). In that case the photoelectron spectrum should correspond to an averaged bent structure, which is highly improbable by virtue of the foregoing discussion. If α is assumed to be the same and $\Delta U^{\ddagger}$ is such that the transition state is appreciably populated (i.e., at least one order of magnitude smaller), the potential energy curve will allow such a large root mean square deviation $\delta\alpha$ in α that only broad, structureless photoelectron bands would be observed, contrary to the measurements.

Therefore the actual potential energy curve must contain one single minimum at 180°. The CNDO/S calculations give total energies which are in agreement with this due to a decrease in nuclear–nuclear repulsion which exceeds the increase of the electronic energy on going to a planar conformation. However, the latter argument cannot be used as evidence for a planar structure regarding the failures of the CNDO/S method.[50] On the other hand, we reject also the argument referring to an oversimplified π-electron concept that, owing to "antiaromaticity", the reduced form of isoalloxazine should be bent.[53]

Now it should be realized that *under the present experimental conditions only we are dealing with truly isolated molecules*. It is, therefore, very likely that environmental forces govern the molecular conformation of the reduced isoalloxazine molecule. This is supported by the presence of hydrogen-bonded dimers in the solid state[39] and it may explain the variety of values[36] found for the dihedral angle α. The high values of $\Delta U^{\ddagger}$, obtained by application of the absolute rate theory,[53] refer to a solvated molecule carrying large substituents. Ascribing these properties to isolated molecules is erroneous.

The dependence of the energy of the highest occupied orbital of a reduced isoalloxazine on the molecular conformation, which in turn is governed by external forces, deserves special attention. It is this "valence orbital" which has to acquire and to lose electrons in the redox reactions, in which the isoalloxazine nucleus participates in biochemical processes. Thus, the apoprotein may influence the "valence-orbital" energy by in-

teraction and, concomitantly, the redox potential of an isoalloxazine prosthetic group associated with it.[53] In addition to protonation, such interactions may explain the large variety of redox potentials of flavoproteins and their further quantification may provide insight into the structure–function relationships in these proteins.

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