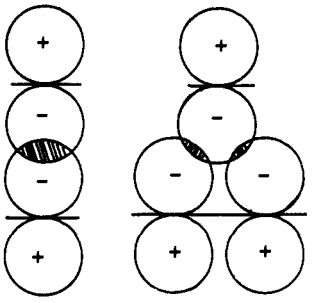
The Nature of the Intramolecular Complex of Flavin Adenine Dinucleotide (FAD)[1]

Sir:

The intramolecular complexing between the two rings in FAD dissolved in polar solvents has been of considerable biological interest. McCormick, *et al.*, have given a number of references relating to the intramolecular complexation,[2,3] proposing model I (Figure 1) on the basis of the HMO electron densities.[4,5] Weber[6] concluded that intramolecular charge-transfer (CT) processes are not important either in the ground or the excited states of the internal complex in FAD. Nevertheless, there is considerable confusion as to the nature of the interaction mechanism and the biological significance of the complexation, partly due to lack of theoretical treatments. Our purpose then is to answer the following pertinent questions: (a) Is charge-transfer interaction in the ground state important? (b) Is charge-transfer state (from adenine to flavin) the lowest excited singlet state? (c) What model is consistent with the bathochromism of 800 cm^{-1} and the hypochromicity of 0.08 for FAD relative to the spectrum of riboflavin? (d) What are the energy and intensity of the calculated charge-transfer transition? (e) Is the complexed FAD biologically significant?

Calculations were carried out for three intercalation-like stacking models (Figure 1) using the π-electron approximation within the restricted Hartree–Fock–Roothaan SCF P–P–P MO scheme[7,8] with 30 singly excited configuration interactions including inter-ring CT configurations. The P–P–P integrals were the same as those satisfactorily used in our laboratory.[9,10] Provision for the interaction between the rings was made using 2p σ-type overlaps.[11]



$$\beta_{rs} = \frac{K}{2} S_{rs}(I_r + I_s)$$

$K = -0.86$

The one-electron core Hamiltonian matrix elements for the 2p σ-type bonding between the two rings stacked by intercalation were evaluated by the Wolfsberg–Helmholz formula[12] ($K = -0.86$[11]).

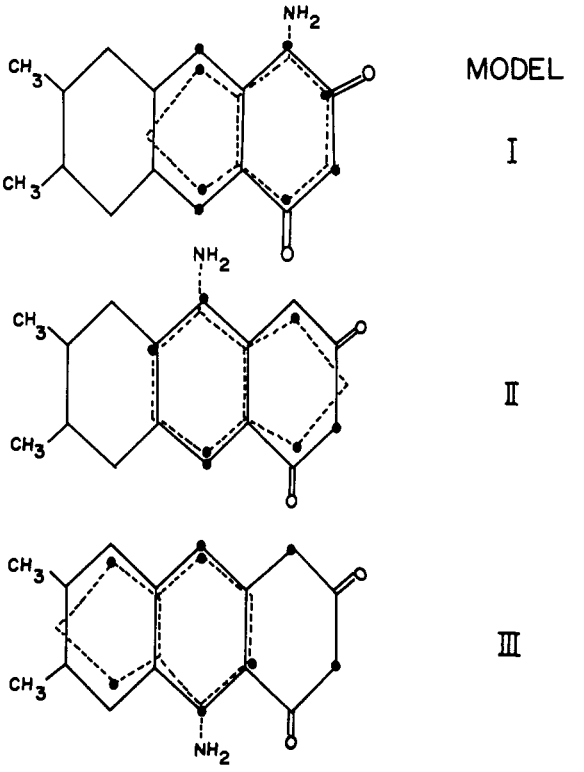


Figure 1. Stacking models for the inter-ring complex of FAD.

The results were obtained at four inter-ring distances: 3.0, 3.3, 3.6, and 3.8 Å. No CT was found at the ground, lowest singlet, and triplet states. The highest CT was predicted to be only 0.017 electron from adenine to isoalloxazine (III) at 3.0 Å. It can be concluded that CT interactions in the ground and lowest excited states of FAD are not important, contrary to the popular notion that the complexing in FAD is of CT character. The absence of the charge transfer can be ascertained from CT valence-bond contributions in the CI matrix. The CI calculations predict that the contribution of the CT resonance form to the lowest excited states of FAD is less than 0.2%.

The lowest CT transition from adenine to isoalloxazine is predicted in the near-ultraviolet region (350–380 nm) for all three models at the 3.0-Å inter-ring separation, with considerable blue shifts (1500–3200 cm^{-1}) at longer distances as expected. However, the oscillator strengths for the CT transitions were 0.001–0.026. Therefore, it is difficult to experimentally identify the CT band under the $\pi \rightarrow \pi^*$ envelopes. In fact, the absorption spectrum of FAD in water is very similar to that of riboflavin, except for the bathochromism (800 cm^{-1} at 445 nm) and hypochromicity of 0.08 in FAD relative to riboflavin. The bathochromism and hypochromicity of FAD can be accounted for in terms of any one of the models at inter-ring separations of 3.3–3.6 Å. A typical example using model I is shown in Figure 2. The lowest transition energy of dimethylisoalloxazine is in complete agreement with the observed transition energy ($\pi \rightarrow \pi^*$, 2.78 eV), when all nonneighbor terms are included. Although the calculations without nonneighbor matrix elements yield somewhat less satisfactory energies, the bathochromism and hypochromicity in the FAD complex are adequately described. Evidently, all three models adopted and

(1) Supported by the National Science Foundation (GB-8055) and the Robert A. Welch Foundation (D-182).

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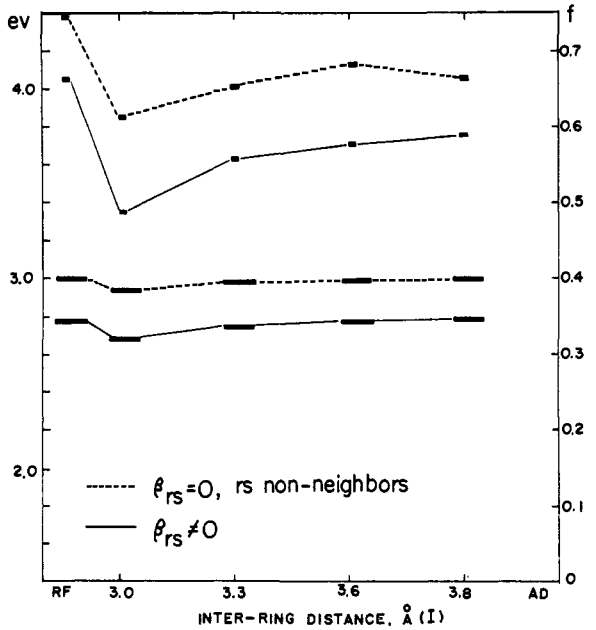

Figure 2. The lowest singlet ($\pi \rightarrow \pi^*$) transition energies (eV) and oscillator strengths (f) as a function of the inter-ring distance (Å) between the flavin (RF) and adenine (AD) moieties of FAD (Model I).

several other models in preliminary calculations did not produce strikingly different spectroscopic quantities. Miles and Urry[13] proposed an FAD stacking model almost identical with model II, based on their circular dichroic data and our calculated transition energies and moments of 6,7-dimethylisoalloxazine.[14,15] The agreement of the observed energies and rotational strengths with those of our calculated data was found to be excellent.[13] The lowest triplet states are also localized in the flavin moiety in all three models. However, sensitive variations of the triplet-state energies with different models were displayed in the calculations. Experimental confirmation of this prediction is attempted.

Most of the flavoenzymes have redox potentials considerably higher than that of free riboflavin ($E_0' = -0.186$ V). FAD in aqueous solution has an E_0' of -0.195 V. Since FAD in aqueous solution assumes the stacked form, it appears that the internal complex of FAD cannot be catalytically important in flavoenzyme reactions with E_0' higher than -0.186 V. We calculated the ionization potentials and electron affinities of all three models on the basis of Koopmans' theorem.[16] Model I is predicted to be a better electron acceptor than II and III, model I yielding an ionization potential and electron affinity higher than those of 6,7-dimethylisoalloxazine. Model II or III, therefore, is consistent with the more negative E_0' of FAD relative to riboflavin.

In conclusion, it is emphasized that all valence electron calculations of FAD are practically impossible. The calculation of the present model is already complex. The estimations of stabilities of the complex models calculated here are not meaningful due to inadequate representation of the dispersion forces in the SCF MO scheme. In spite of the complexity of the system and crudeness of the method, conclusions drawn in this communication are qualitatively significant in view of reasonable agreement between the predicted and experimental quantities discussed above. More detailed accounts of the present work and its extension will be published elsewhere.[17]

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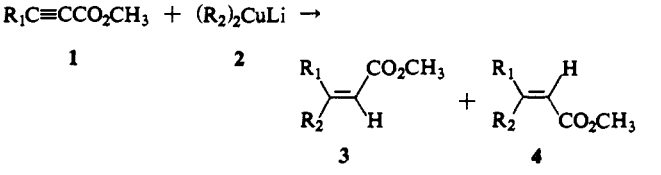
A New Stereospecific Synthesis of Trisubstituted and Tetrasubstituted Olefins. The Conjugate Addition of Dialkylcopper–Lithium Reagents to α,β-Acetylenic Esters[1]

Sir:

The copper-catalyzed 1,4 addition of Grignard reagents to α,β-ethylenic carbonyl compounds is a well-known reaction that has received wide application in synthesis.[2] In contrast there have been only a few studies of conjugate addition as applied to α,β-acetylenic carbonyl compounds.[3] We were intrigued by the possibility of utilizing this reaction for the stereospecific synthesis of trisubstituted olefins, despite the lack of stereoselectivity that had been reported in one case.[3b]

We have found that alkylcopper–lithium complexes (**2**)[2b,4] undergo a facile conjugate addition reaction with α,β-acetylenic esters (**1**), furnishing in high yield the ethylenic esters **3**[5a,b,6] and **4**.[5a,b,6] The copper reagents



(1) For a different new stereospecific synthesis of trisubstituted olefins, see E. J. Corey, J. A. Katzenellenbogen, and G. H. Posner, *J. Am. Chem. Soc.*, **89**, 4245 (1967).
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(5) (a) The assigned structure is entirely consistent with nmr and ir data; (b) a satisfactory carbon–hydrogen combustion analysis was obtained on this compound.
(6) The stereochemistry of the pure isomeric esters **3** and **4** (R_1 = n-C_7H_{15}; R_2 = CH_3) is evident from their nmr spectra. The resonance of R_2 (CH_3) (in CCl_4 solution expressed as parts per million shifts downfield (δ) from tetramethylsilane as internal standard) appears at 1.85 (d, J = 1.3 Hz) in **3** and at 2.15 (d, J = 1.3 Hz) in **4**. Unequivocal evidence of the stereochemistry of **4** was obtained by reduction with aluminum hydride to the corresponding alcohol, 3-methyl-*trans*-2-decen-1-ol, which proved to be identical (nmr, ir, and glpc) with the alcohol obtained from 2-decyn-1-ol by the sequence: lithum aluminum hydride–sodium methoxide reduction, iodination, and methylation with dimethylcopper–lithium (see ref 1).