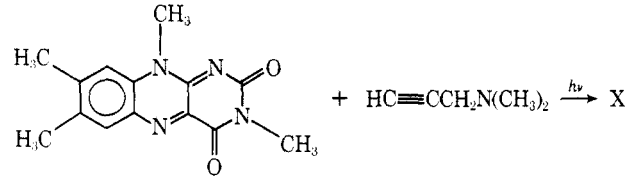
Communications to the Editor

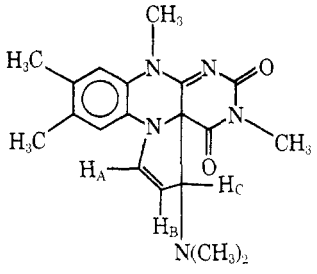
Structure of a Flavoprotein-Inactivator Model Compound

Sir:

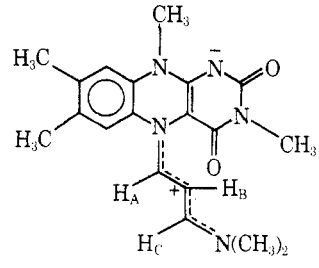
In 1972 Zeller et al. reported[1] the isolation of a new compound, X, from the irradiation of 3-methyllumiflavin in the presence of 3-dimethylamino-1-propyne (Scheme I) and assigned structure I to X based largely upon its NMR spectrum in trifluoroacetic acid. These workers suggested that X was a structural model for the adduct formed when

Scheme I





I



II

monoamine oxidase (MAO) is inactivated by Pargyline-type inactivators, and since then a number of workers[2] have discussed its possible relevance as a model.

In our study of the adduct formed when MAO was inactivated by 3-dimethylamino-1-propyne, we carefully evaluated X as a relevant model compound.[3] We wish to report here that we find the properties of X[4] to be more consistent with structure II than with structure I.[5]

The optical spectrum of X was identical with that previously reported[1] ($\lambda_{max}^{pH\ 3.5}$ 375 (ϵ ~27,000), $\lambda_{max}^{pH\ 8.0}$ 390 nm (ϵ ~23,000)). The intensity of these bands is much higher than expected (ϵ <10,000) for simple N-5 and/or C-4a substituted dihydroflavins.[2a,6] The spectroscopically observed pK between 4 and 6.5 is consistent with the reported[1] pK of 5.3. Substituted dihydroflavins can often be distinguished on the basis of their pK's: simple N-5 substituted compounds usually have pK 6–7 (ionization of N-1) whereas C-4a substituted ones ordinarily have no pK between 2 and 10 (no proton on N-1).[7] The NMR spectrum

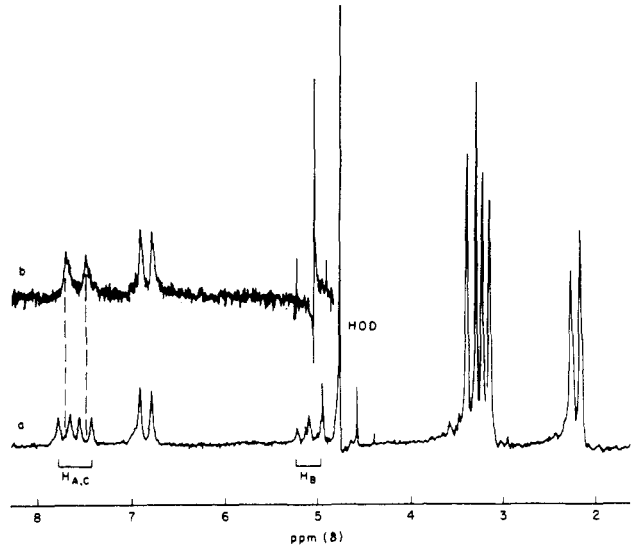
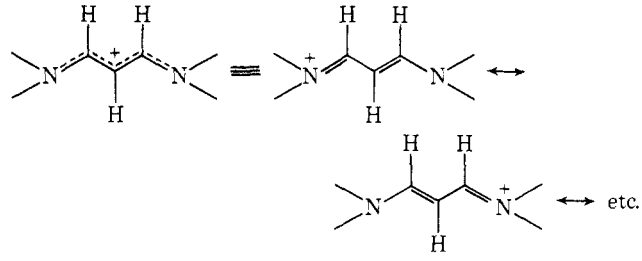


Figure 1. (a) NMR spectrum of X in D_2O. (b) The vinyl region of X in D_2O with decoupling at 5.09 ppm.

of X in CF_3CO_2H was identical with that previously reported.[1] In CF_3CO_2D the "exchangeable" proton at 5.33 ppm disappeared while the multiplet at 7.7 ppm simplified. These data show that the sample of X used for our investigations was identical with that originally described.

Previous assignment of the NMR peak at 5.33 ppm to the enamine-H (H_B of I) means the allylic H (H_C of I) is buried in the multiplet at 7.7 ppm, which seems too far downfield for such a proton.[8] We measured the NMR spectrum in D_2O and obtained a much clearer spectrum (Figure 1) in which the downfield multiplets were nicely resolved.[9]

When the triplet at 5.09 ppm was irradiated, the doublets centered at 7.50 and 7.71 ppm collapsed to singlets (Figure 1). Hence the proton at 5.09 ppm is coupled to each of the two protons at 7.50 and 7.71 ppm which are not coupled to one another. A part structure of the type



would account for the strong deshielding of two of the vinyl protons (H_A and H_C) relative to the third (H_B)[10] and would be consistent with the observed coupling pattern. Since N-1 of the flavin is an amide-like nitrogen, it would not be likely to participate effectively in such resonance stabilization. Furthermore, if N-1 of the flavin were involved, the acid–base properties of the adduct (pK_a = 5.3) would have to be attributed to N-5. Protonation of N-5 at a pH as high as 5 is extremely unlikely since protonation of what should be an even more basic N-5 (that of 1,5-dihydro-3-methyllumiflavin) occurs at pH's below 2.[11] Hence the attachment to the flavin nucleus must be at N-5.

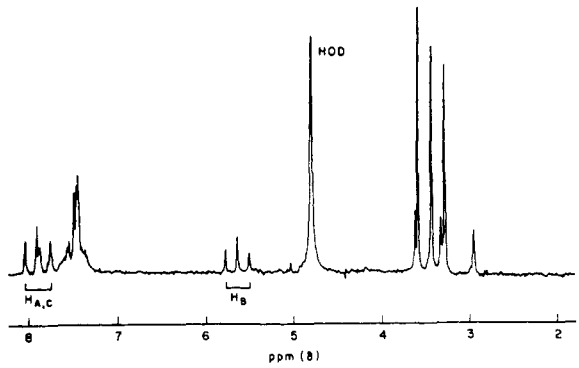
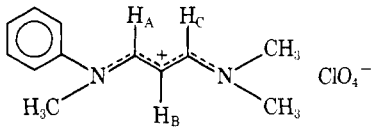


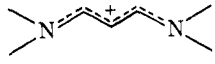
Figure 2. NMR spectrum of IV in D_2O. The three small bands in the *N*-methyl region are probably due to a conformational isomer. These bands could not be removed by repeated crystallization, always appeared in the same ratio, and decreased proportionally to the larger peaks during ozonolysis.

To confirm the NMR assignments, IV[12] was prepared as a model. The NMR spectrum of IV was strikingly similar



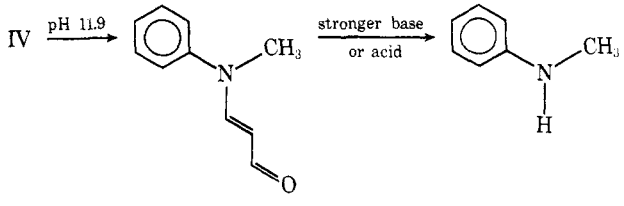
IV

to that of X in both D_2O (Figure 2) and CF_3CO_2H (Figure 3), showing the same coupling pattern and approximately the same chemical shifts for the three vinyl protons. The triplet at 5.55 ppm in CF_3CO_2H disappeared in CF_3CO_2D and the multiplet at 7.8 simplified (Figure 3). As expected[10] for the



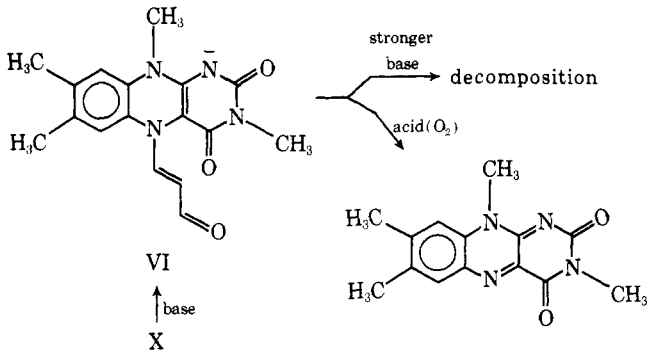
chromophore, IV absorbed very strongly in the uv: $\lambda_{max}^{H_2O}$ 327 (ϵ 45,000).

Both X and IV were stable in acid (pH 1.3 for 11 hr) but labile in base. At pH 11.9, IV was converted to V[13] ($t_{1/2}$ = 5 min). V was in turn converted to *N*-methylaniline in ei-



V

ther stronger base ($t_{1/2}$ = 4 hr in 0.1 *N* NaOH) or in acid ($t_{1/2}$ = 1 hr at pH 1.3). In base, X was converted to a new compound, probably VI,[14] ($t_{1/2}$ = 20 min at pH 11.6).



VI

↑base

X

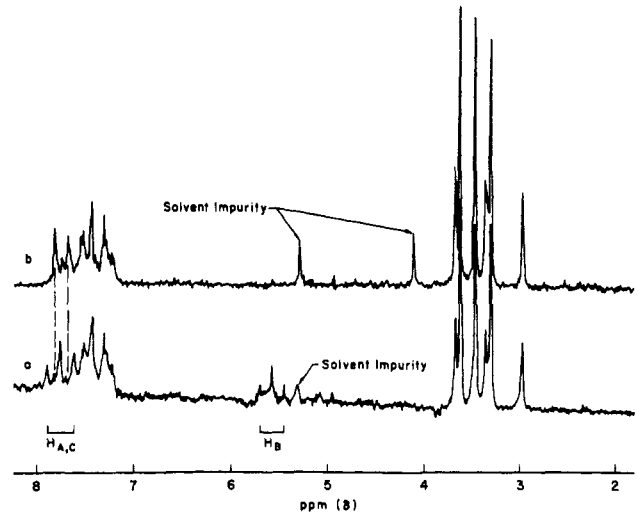


Figure 3. (a) NMR spectrum of IV in CF_3CO_2H; (b) same sample after solvent was replaced with CF_3CO_2D.

VI then underwent decomposition to unidentified products[15] at higher pH, but was converted rapidly to 3-methyllumiflavin in acid, probably by hydrolytic cleavage of the side chain followed by air oxidation of the reduced flavin.

Dimethylformamide (DMF) was identified as a product from both X and IV when each was treated with ozone.[16] Structure I cannot account for this production of DMF from X, but structure II can if ozonolysis of the C_2–C_3 bond occurs. The formation of DMF upon ozonolysis of IV shows that cleavage of the C_2–C_3 bond of II is indeed a reasonable process.

In summary, we find for X that: (1) the pK_a is consistent with those of N-5 monosubstituted reduced flavins; (2) the intensity of the optical spectrum is consistent with a substituted flavin containing extended conjugation; (3) the NMR spectra in D_2O, CF_3CO_2H, and CF_3CO_2D are similar to those of model compound IV; (4) the hydrolytic behavior is similar to that of IV; (5) DMF is a product of ozonolysis. We feel that these data constitute compelling evidence that X has structure II rather than the previously reported structure I. Hence in evaluating X as a model for inhibitor adducts derived from flavin enzymes, this revised structure must be taken into account.

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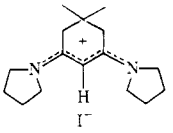
References and Notes

(1) E. A. Zeller, B. Gärtner, and P. Hemmerich, *Z. Naturforsch., Teil B*, **27**, 1050 (1972).
(2) (a) D. J. T. Porter, J. G. Voet, and H. J. Bright, *J. Biol. Chem.*, **248**, 4400 (1973); (b) H. Y. K. Chuang, D. R. Patek, and L. Hellerman, *ibid.*, **249**, 2381 (1974).
(3) R. H. Abeles, A. L. Maycock, J. I. Salach, and T. P. Singer, in preparation.
(4) A generous quantity of X was supplied to us by Dr. P. Hemmerich.
(5) Since completing this work, we have learned from Dr. P. Hemmerich that a similar study has led to the same conclusion: B. Gärtner, Ph.D. Thesis, University of Konstanz, 1974.
(6) (a) S. Ghisla, U. Hartmann, P. Hemmerich, and F. Müller, *Justus Liebigs Ann. Chem.*, 1388 (1973); (b) L. Hevesi and T. C. Bruice, *Biochemistry* **12**, 290 (1973), and references cited therein.
(7) M. Brustlein and P. Hemmerich, *FEBS Lett.*, **1**, 335 (1968); ref 2a and 6a.
(8) For instance, the methylene protons of allylamine in water appear at only 3.75 ppm.
(9) The NMR spectra were measured on a Bruker 90 Fourier transform NMR spectrometer. The chemical shifts are measured relative to water (4.75 ppm) or TMS.

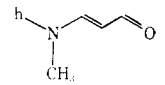
(10) The vinyl proton of



appears at 5.28 ppm and the compound has $\lambda_{max}^{95\%\ EtOH}$ 328 (ϵ 48,700): N. J. Leonard and J. A. Adamcik, *J. Am. Chem. Soc.*, **81**, 595 (1959).

(11) K. H. Dudley, A. Ehrenberg, P. Hemmerich, and F. Müller, *Helv. Chim. Acta*, **47**, 1354 (1964).

(12) Prepared by the methods of H. Bredereck, F. Effenberger, D. Zeyfang, and K.-A. Hirsch, *Chem. Ber.*, **101**, 4036 (1968), and Z. Arnold and A. Holý, *Collect. Czech. Chem. Commun.*, **28**, 2040 (1963), by treating



(see ref 13) with dimethyl sulfate in benzene, replacing the solvent with $CHCl_3$, treating the solution at −60° with dimethylamine, precipitating the product from water with 70% perchloric acid, and recrystallizing it from H_2O.

(13) Prepared by the method of S. M. Makin, A. A. Ishmael, V. V. Yastrebov, and K. I. Petrov, *Zh. Org. Khim.*, **7**, 2201 (1971), by the oxidation of propargyl alcohol with MnO_2 in the presence of *N*-methylaniline.

(14) This tenative structure, previously suggested by Zeller et al. (ref 1), was based on analogy to the chemistry of IV. It is of note that at their respective λ_{max}, $\epsilon_{IV}/\epsilon_V = \epsilon_X/\epsilon_{VI}$.

(15) Cleavage of the C-ring leading to decomposition of IV might occur more rapidly than hydrolysis of the side chain at high pH's. See S. B. Smith and T. C. Bruice, *J. Am. Chem. Soc.*, in press.

(16) Small samples were ozonized to less than completion (by uv spectra), and DMF was detected in the mixtures by NMR. Selective enhancement of the proper peaks occurred when authentic DMF was added to the mixtures.

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Electrocyclic Closure of Bicyclo[4.2.1]nona-2,4-dienes. Photochemical Steering by a Heavy Atom Effect[1]

Sir:

We previously reported that the light-induced isomerization of bicyclo[4.2.1]nona-2,4-diene to the *exo*- and *endo*-tricyclo[4.2.1.$0^{2,5}$]non-3-enes is sensitive to the presence of chlorine substituents.[2] On direct irradiation increasing amounts of the exo isomer are formed when more substituent chlorine is present. We attributed these small, but regular differences to the operation of nonbonded interactions arising between the methylene bridge and the polarizable chlorine atom. However, subsequent experiments revealed that far more subtle factors are at work. We now wish to report that the choice of disrotatory mode followed by the electrocyclic reaction is controlled by the multiplicity of the photoexcited states of the diene and that chlorine, where present, affects the multiplicity by acting as a heavy atom.[3]

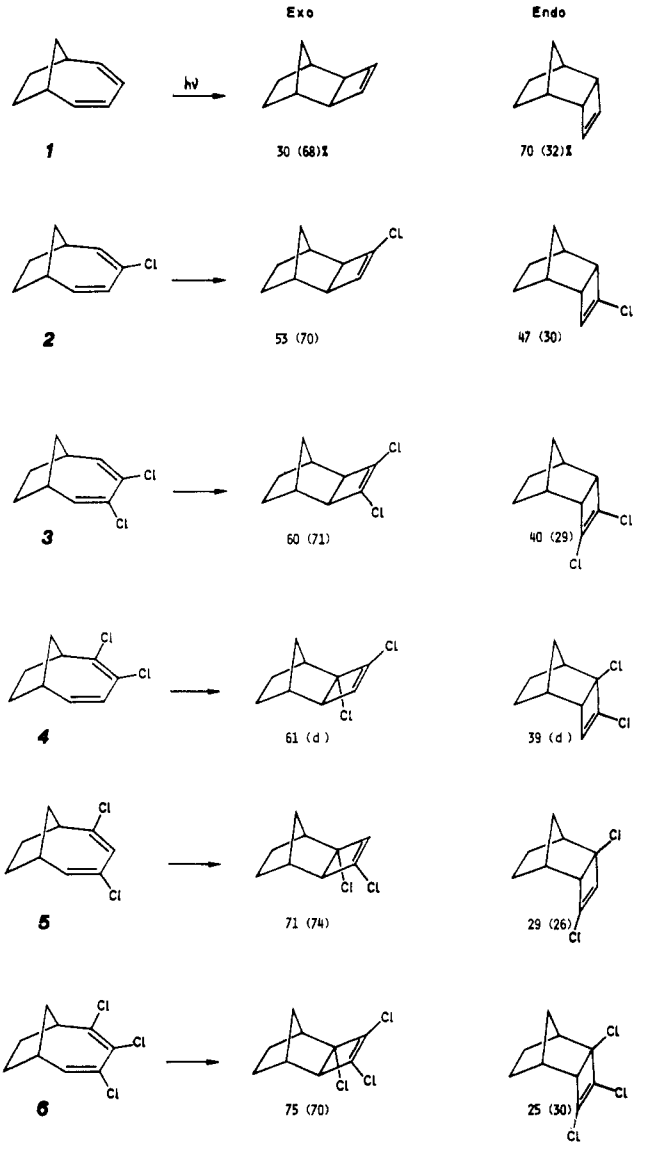
Bicyclo[4.2.1]nona-2,4-diene (**1**) and a series of chloro derivatives, viz., 3-chloro- (**2**), 3,4-dichloro- (**3**), 2,3-dichloro- (**4**), 2,4-dichloro- (**5**), and 2,3,4-trichlorobicyclo[4.2.1]nona-2,4-diene (**6**) were prepared from norbornene by the carbene route.[4,5] These dienes were submitted to direct and sensitized irradiation and, in all cases save one, the electrocyclization occurred to give both the exo and endo tricyclo[4.2.1.$0^{2,5}$]non-3-ene derivatives (Table I).[6]

For direct irradiation, it is seen that the exo–endo ratio depends upon the degree of substitution; the percentage of the exo isomer obtained rises sharply and then gradually with increasing chlorine substitution. The effect is most marked on introduction of the first chlorine atom (cf. **1** with **2**). The influence of a second chlorine substituent is less, but nevertheless reinforces the effect of the first, but more so when placed in a 1,3- rather than a 1,2-disubstituted arrangement (cf. **3** and **4** with **5**). The insertion of a third chlorine atom brings about a correspondingly smaller increase in the amount of exo isomer.

Sensitized irradiation produces a dramatic leveling of the exo–endo ratios. All the bicyclo[4.2.1]nonadienes, the parent hydrocarbon as well as the chloro derivatives, now behave similarly in isomerizing to predominantly the exo isomer. In other words, the macroscopic result is that sensitization is tantamount to chlorine substitution or more precisely disubstitution.

The immediate conclusion which can be drawn is that the sensitized process is a triplet reaction which, regardless of substitution, uniformly proceeds to give a consistent ratio of cyclobutene isomers, but dominated by the exo component.

Table I. Direct[a] and Sensitized[b] Irradiation of Bicyclo[4.2.1]nona-2,4-dienes[c]



[a] Irradiation performed in hexane as solvent, through quartz, with medium pressure mercury lamp, 254 nm, at 25°. [b] Acetophenone or acetone as solvent and sensitizer, irradiation with medium-pressure mercury lamp through Pyrex glass at 25°. [c] Figures in parentheses are the product ratios obtained in the sensitized irradiation experiments, error ±5%. [d] Products decomposed.