MASTER

Technical Progress Report

for

Photo-Initiated Processes in Vision¹

for the period Nov. 14, 1970 to Nov. 15, 1971

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The Principal Investigator has spend approximately 50% of his time on the project. After September 1, Dr. Robert White will be substitute director of the Contract and he should spend ~70% of his time on the project. From September 1, 1971 to June 30, 1972, A. Guzzo will be on leave working in the laboratories of A. Borsellino of the University of Genoa.

I. 1. ESR Study of Model Compounds Related to Visual Pigments

The details of most of our work concerning the radical anions of several polyenals and polyenones are given in

COO-1627-17 "The Radical Anions of Vitamin A Aldehyde and Related Schiff Bases," J. Phys. Chem. Accepted (1971).

COO-1627-19 "ESR Spectroscopy of Non-Cyclic Enones," Mol. Phy. 20, 565 (1971).

COO-1627-20 "Anion Radicals of Non-Cyclic Enones: An Example of Non-Planarity in a Pi System Radical," submitted to Mol. Phy.

COO-1627-21 "ESR Study of Conformational Changes Upon Radical Formation in Non-Planar Systems," submitted to J. Amer. Chem. Soc.

This work is summarized in the section to follow.

Under suitable conditions it was possible to generate moderately stable free radicals of vitamin A aldehyde and of Schiff bases prepared from vitamin A aldehyde. An ESR study of the species in conjunction with numerous molecular orbital type calculations permitted us to make a tentative coupling constant assignment. It was found in the n-butylamine Schiff base that the unpaired electron delocalized over almost the entire polyene chain but had the largest spin density at the Schiff base nitrogen. In order to fit the observed spectrum we were

obliged to neglect couplings to the cyclohexene ring. This was not entirely unreasonable since it is possible that the ring is essentially twisted out of conjugation about the 6-7 bond (see figure below).

In order to test this point we began a study of the α and β -ionones.

α -ionone

β -ionone

Radicals were prepared of these and also the partially hydrogenated and deuterated forms of β -ionone. We were able to assign the angle of

twist in these species by matching the observed coupling constants to the calculated values. The angle of twist in β -ionone was shown to be $\sim 34^\circ \pm 5^\circ$ about the 6-7 bond--a value which if applied to the retinal Schiff base anion would require the cyclohexene ring to be in conjugation.

In order to extract ourselves from this problem a series of Hückel calculations were performed on β -ionone and the homologous series, each member of which increases by one double bond, ending in retinal. It was found that the energy loss which resulted if the cyclohexene ring (i.e. the terminal double bond) were removed from the conjugation was progressively less as one went to longer chained species. Therefore, it is a reasonable argument that in retinal the steric repulsions (which should remain the same in the series) force the ring much further out of conjugation than in β -ionone.

In addition to these findings, we also discovered a new variety of β -coupling entered in considering the coupling constants of non-planar molecules. This was analyzed in the β -ionone radical and the same thinking applied to two other non-planar enones--mesityl oxide and phorone. We postulated that in these small non-planar systems, formation of the anion resulted in increased bond orders about certain bonds such that planar species resulted.

We feel that much fundamental information was obtained in these studies and it has greatly aided our understanding of the polyene Schiff base which has been the model of the visual pigment chromophore.

I. 2. Triplet State Studies

In a previous progress report (COO-1627-11) we reported that the triplet level of retinal had been measured by oxygen perturbation techniques. The final version of this (COO-1627-18) showed that $E_T = 34$ kcal/mole. We attempted to measure the E_T of the series of shorter polyenones and polyenals but no new absorptions were observed with high O_2 pressure. The reason for this failure is not apparent.

The value of 34 kcal for the triplet level agrees quite well with that obtained in COO-1627-13--which was obtained by the energy transfer technique. While energy transfer is supposed to be determined principally by the difference in triplet energies of donor and acceptor, we decided to investigate this for a series of known compounds. In the figure below

No.	Pairs		ΔT_{DA} Kcal/mole	k_{ET} l/mole-sec
	Donor	Acc.		
1	Phenanthrene	Naphthalene ↑ ↓	1.2	0.43
	Biphenyl		5.1	1.0
	Carbazole		9.1	0.30 (.30)*
	Triphenylamine		9.2	2.1
	Aniline		17.0	0.24
	Anisole		19.9	1.3
	Benzoic Acid		20.3	0.76
	Benzene		24.0	0.7
	Carbazole	Biphenyl	4.0	0.08
	Aniline		11.9	0.16

* The value in parentheses is the value of k_{ET} obtained by the Russian Workers.

are given the various k_{ET} s measured for these donor-acceptor pairs (both highly purified) and the separation between their respective triplet states. It appears that $\Delta E_{T, D \rightarrow A}$ is not the only factor influencing transfer. Thus the previous results must have been fortuitous in the choice of donors.

In general this work has provided us with at least one important quantity, E_T in retinal, and some understanding of triplet-triplet transfer. We will digest these most recent findings further before they will be considered for publication.

As our understanding of the factors influencing the triplet state grew, we were able to devise a simple, classical model of electron repulsion which enabled us to predict triplet energies. The accuracy of the method appeared to be ~10-15% and a calculation could be carried out by hand in a few minutes. This is being written up for publication.

II. Continuation Studies

1. Copper in Rhodopsin.

As discussed in a previous progress report, we have evidence that rhodopsin, as extracted by digitonin, may be a cupro-protein. The evidence is in the form of Cu assays by the atomic absorption technique. Rhodopsin solutions were prepared and purified in various ways, some were assayed and to some copper was re-introduced before the assay. Most of this past work was checked and several points were repeated.

We may summarize our findings as follows:

- The retina when assayed whole is rich in copper.
- The copper:rhodopsin ratio is affected by the number of washing steps in the purification procedure.
- The data is consistent with a copper binding site having a $K_{\text{diss}} \approx 2 \times 10^{-6}$.

In the figure below our data for the Cu:Rho ratio is plotted versus the volume of water each sample was exposed to.

Upon addition of known concentrations of Cu^{++} back to the more highly purified solutions, we obtain the data given below.

We may interpret these findings in three ways:

- Rhodopsin is a cupro-protein (1:1), but the copper is leached as the number of washing steps increases.
- Copper can be scavanged by rhodopsin during the course of the isolation.

c) Copper is bound to some other enzyme system which is also extracted by digitonin and is gradually lost as the washings increase.

One last experiment is underway this summer and was made possible by the recent work of Futterman.² We will prepare very highly purified rhodopsin lipid free and re-introduce Copper. The Cu:Rho ratio here should be of further value.

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²M. Zorn and S. Futterman, J. Biol. Chem. 246, 881 (1971).

II. 2. Energy Transfer and Isomerization Studies.

Several interesting features emerge from the state diagram for retinal:

"hot" singlet

"cool" singlet

From the large separation between fluorescence and absorption spectra of the all-trans isomer, very likely absorption of a photon results in the formation of a non-equilibrated or non-relaxed, "hot" singlet. Upon solvent molecule reorientation and slight structural changes in the polyene an equilibrated structure is formed--the "cool" singlet. It is from the "cool" singlet that fluorescence takes place,

however it may be from the "hot" singlet that energy transfer takes place.

If the process of isomerization takes place out of the triplet as has been suggested, then very likely the singlet degrades through an ($n-\pi^*$) singlet to the triplet. We have examined the fluorescence of all-trans retinal and of the n-butylamine Schiff base and found the following:

- a) As the solvent polarity increases ϕ_F (the quantum yield of fluorescence) increases.
- b) If Trichloro Acetic Acid is added to the solution the emission of retinol is observed with high ϕ_F .
- c) If the Schiff base is acidified as above a red shift is observed and no increase in ϕ_F .
- d) The temperature dependence of the ϕ_F seems to be slight with no drastic changes from solvent to solvent.

Observation a) apparently indicates, as expected, that the ($n-\pi^*$) state is affected by solvent polarity and increases in energy. Thus cross over from the ($\pi-\pi^*$) singlet becomes less probable. In TCA the ($n-\pi^*$) state may be removed entirely as follows:

The pi system of the latter species is quite similar to that in retinol, thus the expected emission.

We would argue that in the Schiff base, which has a higher quantum yield than retinal, the (n- π^*) state is not affected by TCA since Blatz³ has shown that the basicity of the Schiff base is quite low and protonation may not occur.

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³P. E. Blatz et al. Photochem. Photobiol. 13, 237 (1971).

Observation d) indicates that some thermal deactivation process occurs in retinal. Its activation energy (assuming we may plot $\log \phi_F$ vs $\frac{1}{T}$) is small ~ 500 cal./mole and may therefore simply be related to the solvent.

We are pursuing studies relating to the state diagram given above. In particular the sensitization studies proposed in the renewal proposal. This should allow some clarification of the mechanism of isomerization of the isomers of retinal and how the quantum efficiency of this process in vivo can be so high.