I. The Photoisomerization of Retinal

II. Iodide-Acetone and Iodide-Acetonitrile Charge-Transfer Complexes

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Approved

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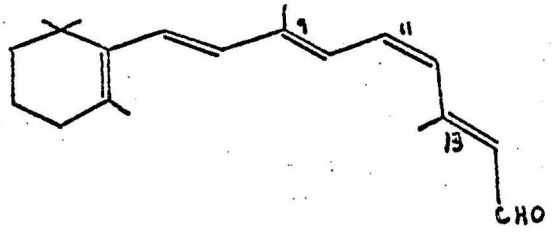
I. The Photoisomerization of Retinal

Introduction

Retinal (vitamin A aldehyde, retinene) is the chromophore in the visual pigment rhodopsin, which is a Schiff's base formed between retinal,

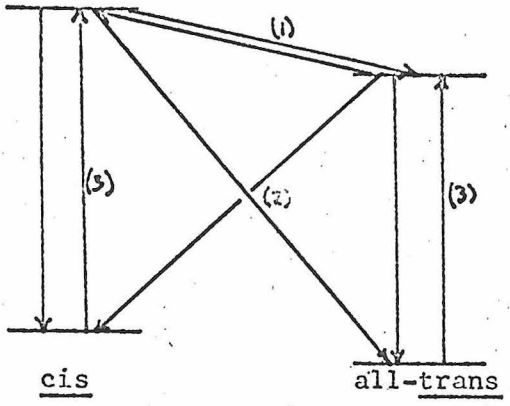


11-*cis* retinal

and the protein opsin. Hubbard and Wald[1] first recognized the importance of the *cis-trans* isomerization in the rhodopsin system. After being struck by a photon, rhodopsin proceeds through several steps, finally producing all-*trans* retinal and opsin. While there is no direct evidence of the initial effect of photon absorption, there are several indications that isomerization from the 11-*cis* to the all-*trans* configuration is most likely.[2,3,4,5] Chief among these is the fact that only 11-*cis* retinal is able to form naturally occurring rhodopsin.[2,3] This isomerization will be of primary importance here.

Generally, the main absorption of the *cis*-isomers falls 50 to 100 Å to the short-wavelength side of the all-*trans* absorption. An explanation of this has been offered:[4] if the geometrical isomers of retinal had a common excited state, the *cis*-isomers should require less energy to reach this than the all-*trans* isomer, since the ground-states of the *cis*-isomers lie at higher energies than that of the all-*trans* (the 11-*cis*, particularly, is sterically hindered and therefore less stable). So the absorption spectra of the *cis*-isomers should be at longer wavelengths than that of the all-*trans*. In fact, however, they lie at *shorter* wavelengths. This

implies that the energy difference between the cis- and all-trans-isomers is still greater in the excited state than in the ground state and that there is therefore no common excited state. Schematically, this would require



The simple existence of the cis absorption to shorter wavelengths than the trans absorption does not necessitate different intermediates, however. There are several problems with the above representation. If one or both of the excited states are singlets, they must be extraordinarily long-lived to allow the necessary time for rotation around the double bond (1) [illegible], or else the Franck-Condon principle must be violated so that the isomerization process occurs in part during the transition(2) If one or both of the excited states are triplets, we are faced with the unlikelihood of a $S_o \longrightarrow T_1$ absorption(3). Neither of these mechanisms is entirely impossible.*

Until definite evidence is obtained for such peculiarities, however, a far better representation involves $S_o \longrightarrow S_1$ absorption and a triplet-state intermediate which may be identical for each intermediate:

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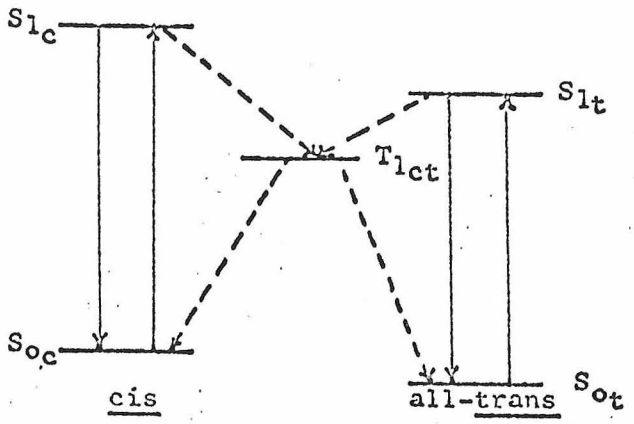
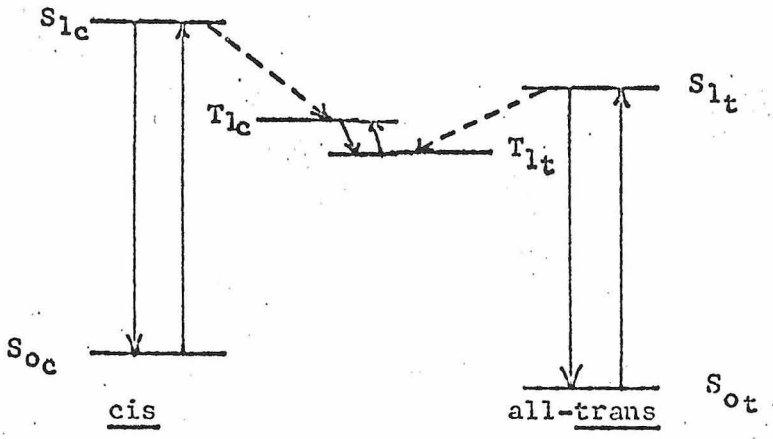
* For example, a lack of conformity with the Franck-Condon principle has been noted in the cis-trans isomerization of the stilbenes.[6] Here, however, a triplet state intermediate (the "phantom triplet") was involved, and the reaction proceeded via triplet sensitizers.



or different:



In the latter case, we note that to avoid violation of the Franck-Condon principle, the cis-trans equilibrium must be established between the two triplets. The relative energies of the two triplets is unimportant, but we also see that in one direction (cis→trans or trans→cis), energy must be "pumped in" to accomplish the isomerization. In view of the fact that rhodopsin (containing 11-cis retinal) and prelumirhodopsin (with all-trans retinal) have been found to be almost perfectly interconvertible by light at -195°, with little or no formation of isorhodopsin (containing 9-cis retinal)[4], it appears that this energy difference is not made up thermally.

At least in vitro, then, we can assume that a triplet mechanism would involve a single triplet intermediate (in vivo, of course, the situation may be quite different).

Virtually no work has been done on the cis-trans isomerization of retinal from a physical point of view. It is the purpose of the work described in this paper to determine whether the isomerization proceeds by a triplet mechanism and if so, what the energy is of the intermediate triplet.

The first stage involves an indirect excitation of retinal via triplet sensitizers to determine the existence of a triplet mechanism. Care must be taken, of course, to choose sensitizers which will not react with retinal and which will not decompose upon irradiation to products (e.g., radicals) which can react with retinal. Although sensitizer triplet-acceptor singlet energy transfer has been observed[7], it is likely to happen only if acceptor $S_o \rightarrow T_1$ transitions are "allowed" but donor $T_1 \rightarrow S_o$ transitions are not "allowed".[8] There is no reason for assuming, a priori, that such complications will arise here.

If a triplet mechanism is established, the next step is to determine the energy of the triplet intermediate. It is not yet known with certainty whether retinal phosphoresces at all. If it does, a phosphoroscope may be used to determine the triplet energy. If not, an estimate might be obtained by looking for a sharp change in the cis/trans stationary-state ratio when sensitizers of different energies are used. The retinal triplet would lie between the higher and the lower sensitizer triplets.

Results

1. The absorption spectrum of retinal in EtOH showed a broad maximum at 3830 Å with $\epsilon_{max} = 45{,}800$, compared to a literature value of $\lambda_{max} = 3810$ Å, $\epsilon_{max} = 43{,}400$.[2] (Hubbard, Gregerman and Wald (9) give $\lambda_{max} = 3830$ Å.)

A spectrum taken of a solution made from retinal crystals which had been allowed to stand exposed to air and occasional light for 48 hours

showed ϵ_{max}=32,200 and λ_{max}=3820 Å, as well as slightly more well-defined "cis-peaks" at about 2500 Å.

When solutions stood exposed to air for 30 hours and then successive minutes of light, lower and lower primary maxima were observed, with a shift of λ_{max} from 3830 to 3800 Å; the cis-peaks also became more and more pronounced at 2570 and 2490 Å. It therefore appears that the photostationary state involves detectable amounts of cis-isomers (perhaps 9-cis and 11-cis at 2490 Å, 13-cis at 2570 Å). This agrees with the results of Hubbard, Gregerman and Wald.[9]

In cyclohexane, the primary maximum was significantly changed: it had a slight shoulder at a wavelength about 100 Å lower than that of the maximum. Here, ϵ_{max} = 43,300, λ_{max} = 3740 Å. The cis-peaks at about 2500 Å were not changed, however. For retinal in C_6H_{12}, ϵ_{2537} = 3920, ϵ_{5000} = 0.

2. The first six sensitizers listed in the "Materials" section were considered for irradiation with visible light (5000 Å). Their spectra were obtained in cyclohexane, benzene, and methanol, depending on their solubility, and all but the following were eliminated because of insufficient absorption at 5000 Å:

Sensitizer	E_T (kcal)
Mesitil	52.6
Duroquinone	51.6
Eosin	43.0

Unfortunately, the arrival of the power source for the mercury-xenon lamp was delayed, and a search was begun for sensitizers with high ϵ at 2537 Å. Of the 72 sensitizers considered, 51 were immediately discarded because they fluoresce (as determined from the literature) in the absorption region of retinal. For initial trials, the following were chosen as having high ϵ_{2537} and low fluorescence yield (benzene, quinoline) or none at all:

Sensitizer	E_T(kcal)	ϵ_{2537}	Reference	% of light absorbed by sol'n* which is absorbed by solvent
Benzene	84.0	220	10	94.08
Pyridine	74.5	2000	11	99.37
Quinoline	62.0	2500	12	99.26
Nitrobenzene	59.9	10000	13	99.84

The sensitizers were used as the solvents. In addition, a 0.04 F solution of retinal in cyclohexane was made up to check the effect of direct irradiation of the retinal at 2537 Å. Controls for all solvents were left unirradiated.

The solutions were irradiated for 35 hours. Only one irradiated solution (quinoline) has been analyzed (by the VPC column described below). No retinal was detected. The solution was so discolored that no retinal could be observed spectrophotometrically, either. The most likely explanation is that the irradiation period was far too long (though stray light from the flame used to seal the degassed ampules and from the source lamp may have had harmful effects). Retinal, with its long conjugated chain, may not stand up very well under hard treatment. On the other hand, the solvent peak, which was rather slow in appearing, may have masked the retinal and/or its decomposition products. The choice between these possibilities awaits the analysis of the remaining irradiated solutions and the controls.

3. Previous methods of analysis for the various isomers of retinal have depended on the change in the absorption spectrum which occurs with isomerization. This is very unsatisfactory, and several types of vapor-phase chromatography columns are being considered. The current candidate is 8.7% Carbowax 20-M, 87.0% Chromasorb P and 12.5% silver fluorborate. With this column, all-trans retinal had a retention time of 1.8 minutes at 140°C and at a helium pressure of 10 p.s.i. Since the retention time was so short, the temperature and the helium pressure were lowered to

* 0.04 F in all-trans retinal.

reduce the substantial column bleeding which occurred and to increase the possibility of separation of the isomers. For the analysis of the irradiated quinoline solution, a column temperature of 105°, an injection block temperature of 145° and a helium pressure of 6.5 p.s.i. were used.

The silver-containing columns may not be satisfactory for our purposes, as they depend on the complexing of the silver with double bonds. There are so many double bonds in retinal that a mere cis-trans isomerization about one of them will probably not make a significant difference in the availability of a complexing site, and there would therefore be little difference in the retention times. A non-silver column which looks particularly useful is that of Blank and Privett.[14] This column (Chromasorb W and 15% ethylene glycol) was used by them to separate the cis- and trans-isomers of methyl lineolate and linolenate.

Experimental

Optics. The light source used was a Hanovia low-pressure mercury arc-lamp. Two filter solutions were used simultaneously: 30 mg p-nitrophenol in 1 liter of water and 1 lb. $NiSO_4 \cdot 7H_2O$ + 300 g $CoSO_4$ in 1 liter of water. The p-nitrophenol solution decomposes rather quickly when irradiated. The following data for the two solutions together give some idea of the spectrum of a fresh solution and a solution after 4 days of irradiation:

	λ, Å	Optical Density
Fresh:		
	>3630	≥2.0
	3500	1.38
	3000	0.95
	2537	0.24
	2500	0.33
	2300	1.85
After 4 days:		
	>3630	≥2.0
	3500	1.00
	3000	0.42
	2537	0.56
	2500	0.59
	2300	1.20

No difficulty is anticipated with the solution here, since it was newly made.

Materials.

All-trans retinal was obtained from Distillation Products Industries and used without further purification. Care was taken to keep the samples evacuated and in the dark. A Safe-light (Wratten, Series 1) by Kodak was used for light during handling.

Sensitizers Naphthacenequinone (from L. Coyne) was sublimed once (hot stage m.p. 290.5-291.5° C (uncorr.)).

Phenanthrenequinone (Eastman Kodak White Label) was recrystallized once from ethanol and sublimed once (hot-stage m.p. 207.5-208.5° C (uncorr.)).

Fluorenone had been zone-refined by Herkstroeter, as described in J. Saltiel's thesis, and was used without further purification.

Eosin had been recrystallized from acetone by L. Coyne and was used without further purification.

Mesitil had been recrystallized by Fry and was used without further purification.

Duroquinone (Aldrich) was sublime once (hot-stage m.p. 111.5-112.5° C (uncorr.)).

Nitrobenzene (Matheson Reagent Grade) was steam-distilled with 6 N H_2SO_4, collected, dried over $CaCl_2$ and vacuum-distilled; a middle fraction was collected.

Pyridine (Baker Reagent Grade) was distilled once over BaO; fraction collected: 113.7-114.0 (uncorr.).

Quinoline (Eastman Practical Grade) was distilled twice under reduced pressure, once from zinc dust and once from a mixture of zinc dust and BaO. A middle fraction was collected each time.

Benzene had been washed with conc. H_2SO_4 and distilled over P_2O_5 by L. Coyne.

Cyclohexane (Eastman Reagent Grade) was washed twice with a 1:1 HNO_3-H_2SO_4 mixture, twice with H_2SO_4 and three times with water. It was then dried over $CaSO_4$ and passed through a 16 cm silica gel column.

Column Packing Chromasorb P (Johns-Manville) was base-washed by P. Wagner.

Silver fluorborate (Ozark-Mahoning Co.) was used without further purification.

Carbowax 20-M was used straight from the bottle.

Solvents

Benzene (Matheson Spectroquality) was used without further purification.

Methanol (Matheson Spectroquality) was used without further purification.

Cyclohexane (Matheson Spectroquality) was used without further purification.

Ethanol (100%, from stockroom) was used without further purification.

Dichloromethane (Matheson Reagent Grade) was used without further purification.

Tetrahydrofuran (technical grade) was dried over anhydrous sodium sulfate and distilled over sodium metal; fraction collected: 64.9-65.4° C (uncorr.).

Spectra. All spectra were obtained on the Cary 14 recording spectrophotometer at room temperature. Matched 1 cm fused silica cells were used. Spectra were obtained of the benzene, methanol, cyclohexane and ethanol used as solvents, and none showed any impurities.

VPC Column. 25 g Carbowax 20-M was dissolved in dichloromethane and poured over 250 g Chromasorb P to make a slurry. The dichloromethane was evaporated on a Rinco roto-evaporator. 12.5 g silver fluorborate was dissolved in THF and added to the carbowax-chromasorb mixture to form a slurry; this was dried on the roto-evaporator.

Over a period of hours, the mixture turned dark, indicating that perhaps not all of the dichloromethane had been taken off and had formed a small amount of silver chloride in the mixture. In any event, a portion of the mixture was packed into a three-foot stainless steel column. The instrument was a Loenco Model 15B, with a thermal detector.

II. Iodide-Acetone and Iodide-Acetonitrile Charge-Transfer Complexes

Iodide-acetone and iodide-acetonitrile charge-transfer complexes have been shown to exist, and their heats of formation are being determined.

Hammond and Gray suspect charge-transfer complexes of lowering reactivity in nucleophilic additions by I^- in acetone and acetonitrile.

In flash-photolysis experiments, Edgerombe and Norrish have pointed to the existence of an excited iodide charge-transfer complex as the intermediate in the formation of I_2^- in H_2O and CH_3CN:

$$(I^-, H_2O) \xrightarrow{h\nu} (I^-, H_2O)^*$$
$$(I^-, H_2O)^* \longrightarrow I + H + OH^-$$
$$I + I^- \longrightarrow I_2^-$$

The reaction

$$(I^-, CH_3CN)^* \longrightarrow I + CH_3 + CN^-$$

is inferred from the proven existence of CH_3I, presumably formed by

$$I + CH_3 \longrightarrow CH_3I.$$

Jortner, Ottolenghi and Stein have shown the existence of I atoms in solvents like alcohols and CH_3CN in the primary photochemical stage.

Results

Absorption spectra of KI and NaI in acetone and acetonitrile were obtained. 10^{-4} F solutions of these salts in acetonitrile showed strong maxima at:

NaI: 2070, 2465 Å
KI: 2070, 2465 Å.

Iodine solutions were also made up to make sure that the maxima were not due to iodine-complexes formed upon oxidation of the iodide:

I_2 in acetonitrile: 3625, 2910 Å (both very broad).

When the iodine solution was allowed to stand in the sunlight for several minutes, sharp maxima appeared at 2490 and 2570 Å (these may be due to iodates). Thus the maxima observed in the iodide-acetonitrile solutions are

indeed due to the presence of iodide and not to some other species.

10^{-3} F solutions of NaI and KI in acetone exhibited very strong maxima at:

KI: 2155 Å
NaI: 2150 Å

and a shoulder at:

KI: 2390 Å
NaI: 2390 Å.

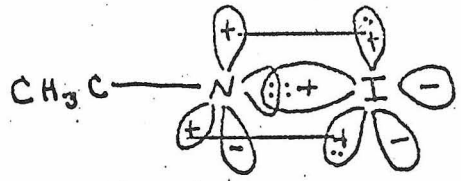
At 10^{-4} F, distinct peaks were revealed:

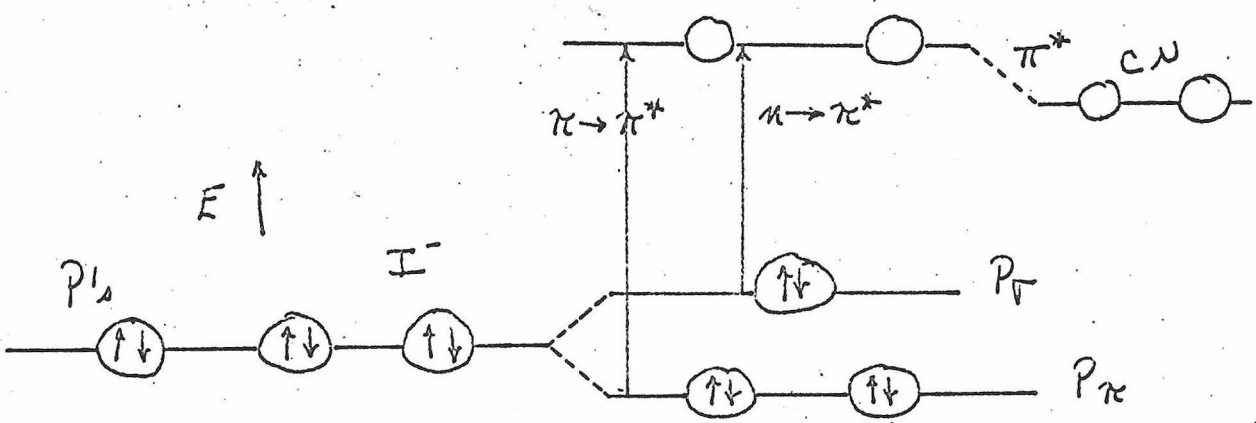
KI: 2160, 2410 Å
NaI: 2160, 2410 Å.

For acetone, the programmed spectrophotometer slit is wide open in the vicinity of the higher-wavelength maximum, and the wavelength recorded is probably not very accurate. The lower-wavelength maximum is correct, however, as the slit is once again 0.1 mm.

Discussion

According to Dr. Gray, these maxima indicate the existence of "low-energy $I^- - \pi^*$ charge-transfer". One of the possibilities for CH_3CN is





Work is now under way to determine the heats of formation of the complexes.

Experimental

MATERIALS. (All used without further purification.)

Acetone--Matheson Spectroquality

Acetonitrile--Matheson Spectroquality

Cyclohexane--Matheson Spectroquality

Sodium Iodide--Baker Reagent Grade

Potassium Iodide--Baker Reagent Grade

Spectra. Again, all spectra were obtained on the Cary 14 recording spectrophotometer. The cells used for the acetonitrile solutions had a path-length of 1 cm. It was not possible to dilute the acetone solutions with enough cyclohexane to decrease the absorption of the solvent sufficiently in the UV because of the precipitation of the salts. Therefore, matched 0.1 cm fused silica (#S18-120) cells were obtained from Pyrocell Manufacturing Co. and used for the acetone solutions.

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