Comprehensive Investigation of the Spectroscopy and Photochemistry of Retinals. II. Theoretical and Experimental Consideration of Emission and Photochemistry

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Abstract: *all-trans-*, 9-*cis*-, and 13-*cis*-retinals exhibit a fluorescence at 77°K in a rigid matrix which is quenched in viscous or fluid solutions at higher temperatures. The 11-*cis*-retinal shows no fluorescence ($\leq$0.001) from 77 to 298°K. The quantum yields of fluorescence are dependent upon the wavelength of excitation. The fluorescences are assigned as π,π^*. It is possible that the lowest excited singlet state in 11-*cis*-retinal is S_{n,π^*}. Photoisomerization does occur for the cis isomers at 77°K and higher temperatures. Theoretical calculations have been carried out including doubly excited state configurational interaction. Potential energy curves have been generated as a function of angle of twist around certain double bonds. These are consistent with the emission and photoisomerization results. Among other predictions based on the potential energy curves, cis → trans photoisomerizations are expected to have considerably lower activation energies than trans → cis. This has been experimentally verified. The observed triplet–triplet absorption has been assigned as the $T_1 \rightarrow T_7$ transition.

There has been limited study of either the emission spectral properties or theoretical aspects of the retinals.[2-10] Fluorescence of *all-trans*-retinal has been reported with a maximum at ~509 nm at −196° whereas no or extremely little fluorescence exists for the 11-*cis*-retinal.[2] There has been an investigation of the emission characteristics of isomeric retinols and some other of the *all-trans*-retinyl polyenes, but not the aldehyde (retinal).[4] All of the retinols exhibited fluorescence but no phosphorescence. Very recently a study was made of the emission spectroscopy and theoretical properties of acrolein (a vinyl aldehyde).[11] Of particular importance was the determination of the shape of the potential energy curves as a function of angle of twist around single and double bonds and the significance of these relative to the emission and photochemical behavior.[11] These aspects are of concern in the retinals as well (*vide infra*).

Although no phosphorescence has been seen from the retinals, triplet–triplet absorption has been observed in fluid solution for *all-trans*-retinal at 450 nm with a lifetime of 10^{-5} sec.[12,13] The quantum yield of triplet occupation (Φ_{IS}) is reported to be 11%.[13] Based on energy-transfer experiments, the lowest triplet state has been assigned to be 38 kcal above the ground state (0–0 in absorption at 880 nm).[14] Rhodopsin has been reported to show a very weak fluorescence at both low (−196°) and near room temperature (3°) at ~570 nm.

The present paper considers a comprehensive investigation of the fluorescence properties of the isomeric retinals including quantum yields, temperature and wavelength dependence of the quantum yields, theoretical calculations of the potential energy curves for the ground state and excited states as a function of angle of twist around single and double bonds, and an interpretation of the spectral and photochemical properties of the retinals.

Experimental Section

The retinals were obtained from the sources noted in part I. The absorption spectra were obtained in the manner described in part I and the solvents were purified as in part I. The Lexan film samples were prepared in the same manner as that described in part I. Emission and excitation spectra were determined from an emission apparatus with a front-face-illumination and detection configuration as described by Becker, *et al.*;[15] the curves are shown uncorrected. However, the quantum yields of emission as a function of exciting wavelength are corrected for all experimental parameters and calculated as that described by Becker, *et al.*[15]

It is worth noting that in particular 11-*cis*-retinal must be handled with utmost care. We have obtained emission from 11-*cis*-retinal when it was not obtained from newly opened sealed bottles, when the crystals appeared wet, and when multiple dilutions were done, especially in room light.

The quantum yields of fluorescence were determined at two wavelengths in degassed solutions using 9,10-diphenylanthracene (9,10-DPA) as a reference. The absolute fluorescence quantum yield of 9,10-DPA has been found to be 1.0.[16] A standard and the unknown were excited under the same conditions for each quantum yield determination and the areas under the emission curve were measured and compared. The emission of Rhodamine B was used to calibrate the excitation sources at various wavelengths and to determine the effect of varying the slit widths and electronic attenuation. If possible, the slit widths and wavelength of excitation light were kept constant and only the electronic attenuation was varied. The intensity of emission was corrected for phototube response by

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using the manufacturer's published sensitivity curve. Several compounds were used to check the accuracy and precision of the method. For benzophenone phosphorescence, a value of 0.74 was obtained for ϕ_P at 77°K, compared to the absolute quantum yield of 0.75 obtained by Gilmore, *et al.*[17] (Parker and Hatchard[18] obtained a yield of 0.71 compared with $\phi_F = 0.54$ for fluorene.) Rhodamine B in 95% EtOH at 25° gave a value of 0.98 for ϕ_F compared to the absolute quantum yield of fluorescence of 0.97 obtained by Weber and Teale.[19] The fluorescence yield of fluorene at 25° in 3-MP was found to be 0.85, compared to 0.80 obtained by Berlman (referenced to $\phi_F = 1$ for 9,10-diphenylanthracene)[20] in a hydrocarbon solution at this same temperature. It is seen from the above examples that our method gives values in excellent agreement with absolute quantum yield values (±2%). The deviation in any of the cases is less than ±6% from the best published values. Because front-face excitation is used and the path length of absorption is only 2 mm, errors due to self absorption are minimized. Successive determinations on the same sample usually gave values differing by no more than ±2% and never more than ±5%. Because of the excellent agreement of our values of quantum yields for compounds for which there are absolute quantum yields, we believe that our quantum yields are the equivalent of absolute values.

Assuming the independently measured value of the quantum yield at 400 nm was correct (using the procedure in the preceding paragraph), the quantum yield at 380 nm was predicted based on the excitation spectrum and corrections for all experimental parameters.[15] The predicted value at 380 nm was within 5% of the value determined independently by the method in the preceding paragraph. This provided assurance that the procedure utilizing an excitation spectra and corrections for experimental parameters[15] gave correct relative quantum yields as a function of wavelength. Thus, in turn, the actual quantum yields at all wavelengths can be determined based on an independent determination at any one wavelength (or more if desired).

Theoretical Calculations

The model used for the calculations is the same as that given in part I, Figure 1. For the planar molecule (0° twist), the ground-state and excited-state energies were calculated utilizing a Pariser–Parr approximation[21] for the repulsion integral without the bond order correction (see part I). The β_{C-C} and $\beta_{C=C}$ for the planar molecule (all-trans, 11-cis, 9-cis) was the same as that used in part I (without bond order correction) to calculate the state energies. The $\beta_{C=O}$ for the carbonyl group was obtained by Kon's equation.

For the twisted molecules, the $\beta_{C=C}$ for the twisted bond was calculated using

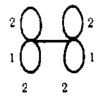
$$\beta_{C=C}(\theta) = \beta_{C=C} \cos \theta \qquad (1)$$

where θ is the angle of twist and $\beta_{C=C}$ is the value for the planar structure (0° twist). The $\beta_{C=C}$ and β_{C-C} for all other bonds was the same as those used above. The usual electron repulsion integral approximations such as Pariser–Parr[21] and Mataga–Nishimoto[22] depend on atomic distance, but not upon the angle between the p atomic orbitals. Since we wished to calculate potential energy curves as a function of angle of twist around the 11–12 and 9–10 double bonds of retinal, the general utility of the conventional procedures (*vide supra*) for determining the repulsion integrals was evaluated. This was done by calculating the potential energy curves for twisted ethylene using the Pariser–Parr approximation for the repulsion integrals within the general SCF–MO–CI method. A differently calculated potential energy curve of the triplet state[23,24] is in harmony with experimental results on the photoisomerization of *cis*- and *trans*-1,2-dichloroethylene.[25] To produce such potential minima in the excited states of ethylene at 90° (within the framework of the semiempirical SCF–MO–CI with zero differential overlap using the point-charge atomic repulsion integral), the following function was used for the atomic repulsion integral of the atoms of the double bond of interest[26]

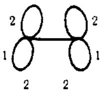
$$\gamma_{C=C}(\theta) = \gamma_{C=C}(0) + A(1 - \cos \theta) \qquad (2)$$

where the first term corresponds to the planar atomic repulsion integral value, θ is the angle of twist, and A is a parameter. All other atomic repulsion integrals $[\gamma_{C=C}(0)]$ were calculated by the Pariser–Parr equation. Calculations using this approach were carried out for the retinals using the Pariser–Parr approximation for all $\gamma_{C=C}(0)$ and included all singly excited configurations.

The twisting of a bond results in nonparallelism of the *nonadjacent* p atomic orbitals and therefore the introduction of $2p\sigma$ components in the atomic repulsion integrals. This effect will make an atomic repulsion integral value larger than the one with only a pure $2p\pi$ component corresponding to the same distance. To take this effect into account, an off-center position was assumed for the location of the center of the atoms involved along the $2p\pi$ atomic orbital axis.[26,27] The off-center distance was looked upon as a parameter.



$$\sigma_u = \sum_{i=1}^{2} \sum_{j=1}^{2} 1/r_{ij}$$



$$\sigma_t = \sum_{i=1}^{2} \sum_{j=1}^{2} 1/r_{ij}$$

The value of the repulsion integral $\gamma(r_{ij})$ for the untwisted case at separation r_{ij} is calculated by usual Pariser–Parr method. For the twisted case

$$\gamma(\theta,\Phi,r) = (r_{ij})\frac{\sigma_u}{\sigma_t} \qquad (3)$$

All of the foregoing calculations involved complete singly excited state configurational interaction.[28,29] It is known that the inclusion of doubly excited state configurational interaction has a notable effect on state energies.[26,30-33] In addition, the shape of the potential energy curves resulting from twisting around a double bond are affected.[30] In order to take into account all

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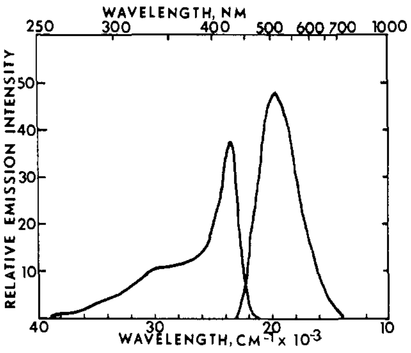

Figure 1. Emission and excitation spectra of *all-trans*-retinal in 3-methylpentane at 77 °K.

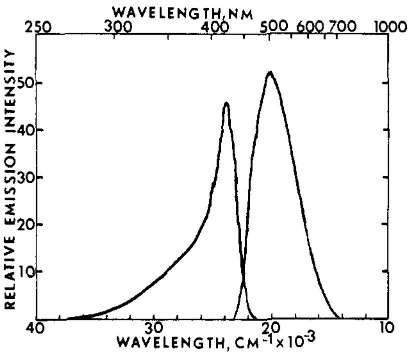

Figure 2. Emission and excitation spectra of 9-*cis*-retinal in 3-methylpentane at 77 °K.

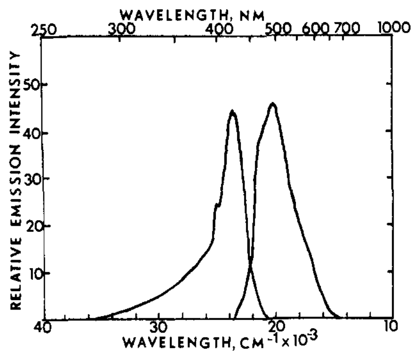

Figure 3. Emission and excitation spectra of 13-*cis*-retinal in 3-methylpentane at 77 °K.

singly and doubly excited configurations for a retinal, the order of the secular equation becomes 478 and 477 for singlet and triplet states, respectively (36 arise from all singly excited configurations). Because of computer memory storage and computational time, a calculation of the matrix elements (477 × 477 or 478 × 478) could not be done. Consequently, we considered the three highest filled and lowest four empty orbitals to reduce the secular equation to 97 and 96 for the singlet and triplet states, respectively. Thus, there is complete singly and partial doubly excited state configurational interaction. This choice will represent a major part of the total interaction expected if all singly and doubly excited states were considered.

Results and Discussion

Table I gives the fluorescence maxima for the *all-trans*-, 9-*cis*-, and 13-*cis*-retinals at 77 °K in 3-methylpentane. We observe essentially *no* emission ($\phi_F \leq 0.001$) from 11-*cis*-retinal under the same conditions. This is in agreement with our earlier work on a different sample.[2] In addition, there was some indication of a very weak fluorescence from a high concentration of *all-trans*-retinal at room temperature in Lexan with a maximum at ~540 nm. This was some 30–40 times weaker than that obtained at 77 °K. In fact, no excitation spectrum could be obtained at room temperature. In addition, no emission was obtained from the pure solid *all-trans*-retinal at room temperature.

Table I. Fluorescence of Retinals at 77 °K

Compound	Maximum, nm[a]
All-trans[b]	508–510
9-Cis	519–521
13-Cis	503–506

[a] In 3-methylpentane. [b] All-trans at 512–513 nm in EPA at 77 °K, 525–530 nm in glycerol–methanol (85–15%) at 198 °K, and ~528 nm in Lexan at 77 °K.

We were unable to detect any phosphorescence for any retinal to 8200 Å despite extensive effort. Based on energy-transfer experiments,[14] the 0–0 band of phosphorescence of *all-trans*-retinal is estimated to be at ~880 nm.

It is important to comment concerning the effect of temperature upon the fluorescence intensity of the 9-*cis*-, 13-*cis*-, and *all-trans*-retinals. When the temperature of the 3-methylpentane solution is raised from 77 to 85–90 °K where a soft glass exists, the quantum yield of fluorescence decreases to 2–10% of its value at 77 °K. If the temperature is further increased to 100–120 °K where the solution is highly viscous, the fluorescence is no longer detectable ($\phi_F \leq 0.001$). The fluorescence is also not detectable at the higher temperatures of 198 and 298 °K, where the 3-methylpentane solution is fluid. A glycerol–methanol solution (85–15%) is a rigid glass at 198 °K. *all-trans*-Retinal has a fluorescence intensity in this solution at 198 °K of approximately one-half of that in 3-methylpentane at 77 °K. Recall that no fluorescence is detectable in a fluid 3-methylpentane solution at 198 °K.

Figures 1–3 show the fluorescence and excitation spectra for the three retinals that do fluoresce. Table II presents the quantum yields of fluorescence as a function of the wavelength of excitation for *all-trans*- and 9-*cis*-retinals in 3-methylpentane at 77 °K.

We have referred to the emission from the 9-*cis*-, 13-*cis*-, and *all-trans*-retinals as fluorescence. This primarily is based upon the overlap of the onsets of the absorptions and excitation spectra with those of the emissions and the mirror-image relationship of the emissions and the long-wavelength absorption bands. Similar criteria were used for the earlier results from the all-trans isomer.[2] Another important question is the assignment of the state from which the emissions originate. We assign the emissions as originating from the lowest excited S_{π,π^*} state. This is based on the following facts. The mirror-image relationship is with reference to the longest wavelength band, which certainly results from a transition to the lowest S_{π,π^*} excited state. Secondly, the quantum yields of fluorescence are

Table II. Quantum Yields of Fluorescence of Some Retinals at 77°K[a]

Wavelength of excitation, nm	Φ_F	
	All-trans	9-Cis
320	0.20	0.013
340	0.18	0.011
360	0.17	0.010[b]
380	0.21[b]	0.011[b]
400	0.29[b]	0.011
410	0.38	0.012
415	0.45	
420	0.56	0.035
425	0.64	
430	0.70	0.094
435	0.68	
440	0.71	0.163

[a] See Experimental Section for general procedures. [b] Quantum yields at these wavelengths individually determined by the procedure in the Experimental Section. All other values determined from the excitation spectrum based on the absolute value at the two wavelengths denoted.

generally high, which is *not* expected when the state origin is S_{n,π^*}.[34] A calculation[8] as well as other considerations[8] place the lowest S_{n,π^*} state below the lowest S_{π,π^*} state. However, this is not in agreement with experiment. Several factors can modify the conclusion that the S_{n,π^*} state is lowest. Approximations used in calculation can easily result in errors larger than the predicted small energy differences (0.05–0.1 eV) between these states. In addition, even though the S_{π,π^*} state is lowest, spin–orbit coupling and vibronic coupling among singlets and triplets can be a dominant mechanism connecting singlet and triplet states.[34] This mechanism should be quite important in the retinals, considering especially the expected small energy gap between the S_{π,π^*} and S_{n,π^*} states. Thus, we agree that the lowest triplet is T_{π,π^*}.[13] On the other hand, the lowest singlet is assigned as S_{π,π^*} (not S_{n,π^*}) and the mechanism of intersystem crossing involves spin–orbit and vibronic coupling for 9-*cis*-, 13-*cis*-, and *all-trans*-retinals.

In the case of 11-*cis*-retinal, the fluorescence is essentially absent ($\Phi_F \leq 0.001$). This result is not only different from that of the all-trans case but also from the results obtained for the other cis isomers (*vide supra*). Two major possibilities could explain these results. First, in the case of the 11-cis, the S_{n,π^*} state could be the lowest excited singlet state. In this instance, it would be expected that fluorescence would be essentially quenched and, concurrently, the probability of intersystem crossing would be expected to be high. Secondly, the S_{π,π^*} could remain lowest but a combination of increased intersystem crossing (through spin–orbit and vibronic coupling between the close-lying S_{n,π^*} and S_{π,π^*} states) and increased cis → trans photochemistry would quench the fluorescence. The spectroscopic data on the 9-*cis*- and 13-*cis*-retinals are consistent with the assignment of the S_{π,π^*} state as the lowest state (*vide supra*). However, cis → trans photochemistry is apparently not sufficiently rapid to totally quench the fluorescence. Based on the shape of the potential energy curves (*vide infra*), we would not expect the rate constant of cis → trans isomerization to vary significantly among the cis isomers. However, the 11-cis isomer has an inherent relatively significant steric interaction (CH_3–H) which will result in twisting within the molecule and an increase in the S_{π,π^*} state energy especially at small angles (at the cis side); also see part I. Thus, the potential energy curve could be modified such that a very low barrier would exist in T_1 and that present in S_1 would be further reduced. This could modify the cis → trans isomerization rate constant. The reason for the S_{n,π^*} state possibly being lowest also would result from the fact that near small angles the S_{π,π^*} state would be increased in energy such that S_{π,π^*}–S_{n,π^*} state interchange could occur. It is not possible with the data presently available from all sources to unequivocally decide which mechanism is operating.

Before proceeding to the theoretical aspects, we wish to make some observations regarding the photochemical behavior of the retinals. Earlier[2] we noted that irradiation of *all-trans*-retinal at 77°K resulted in the slow formation of a photoproduct (*vide infra*) having an absorption band maximizing in the 300-nm region (emission maximum at ~460 nm). Upon warming the solution to room temperature and recooling to 77°K, the original retinal spectrum returned, with a concomitant disappearance of the band at 300 nm. Continuous irradiation into the 300-nm band also gave parallel changes with additional buildup of a shoulder on the long-wavelength edge of the 380-nm band of retinal. The shoulder on the long-wavelength edge of the 380-nm band also disappeared on warming to room temperature. We have repeated a portion of these experiments and find similar results and 85–90% reversibility. In addition, the first absorption band is slightly blue shifted and the cis band in the 250-nm region is increased. These results indicate that some trans–cis isomerization has occurred. The photodecrease and subsequent thermal increase of the intensity of the 380-nm band is parallel to results obtained by others.[35]

Irradiation of 13-cis and 9-cis (1-kW xenon arc at ~400 nm with 2-mm slits through a monochromator) at 77°K results in a decrease of the fluorescence intensity. In both cases, a photoproduct similar to that from all-trans is formed based on the buildup of a new emission band with a maximum at ~460 nm.[2] In the case of the 9-*cis*-retinal, the emission maximum red shifts (albeit decreases) to near that of *all-trans*-retinal. These data indicate that both a photoproduct and cis–trans isomerization are occurring in the case of the 9-cis isomer. Irradiation of 11-*cis*-retinal under conditions as noted above (for ~1 hr) ultimately resulted in a decrease in the intensity of a fluorescence. This alone indicated little isomerization to all-trans and formation of photoproduct. However, if we irradiate 11-*cis*-retinal at 77°K with a 1- or 2.2-kW xenon through filters with a band pass of 350–520 (maximum ~380 nm or a cutoff at 380 nm), we detect an initial increase in the intensity of long-wavelength band maximum and a decrease in the cis band. That is, in 20 sec the absorbance of the long-wavelength band maxima increased ~10% and the intensity of the cis band decreased ~13%. Assuming only all-trans is formed, this represents approximately a 35% conversion. Also, the intensity of fluorescence increases. These results indicate the formation

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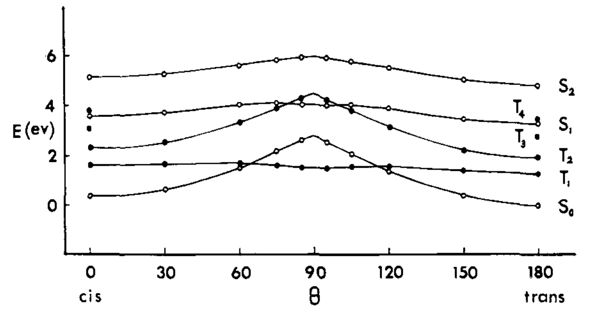


Figure 4. Potential energy curves for twisting around the 11–12 double bond employing eq 1, 2, and 3 and complete singly excited state configurational interaction: ○, singlet states; ●, triplet states.

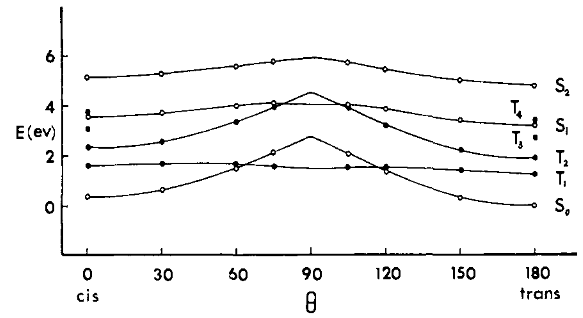


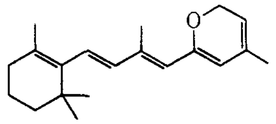
Figure 5. Potential energy curves for twisting around the 9–10 double bond employing eq 1, 2, and 3 and complete singly excited state configurational interaction: ○, singlet states; ●, triplet states.

of *all-trans*-retinal from the 11-cis isomer. Continuation of pulses of irradiation ultimately results in a decrease of the intensity of the long-wavelength band maximum to below that observed for 11-*cis*-retinal, indicating formation of a photoproduct. For 13-*cis*-retinal, 20 sec of irradiation with light beyond 380 nm causes an increase in absorbance and slight red shifting of the long-wavelength band maximum. Also, the emission intensity increases. Continued irradiation for a total of 30 min results in the retinal emission decreasing but emission at ~460 increases. These results indicate the initial formation of *all-trans*-retinal from 13-*cis*-retinal, and continued irradiation causes the formation of photoproduct (see later discussion).

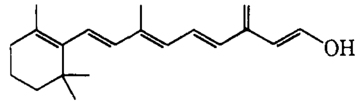
Irradiation of any of the retinals at room temperature (1-kW xenon arc through a filter passing 240–400 nm with maximum at ~330 nm) results in essential total destruction in a short time. On the other hand, for 11-*cis*-retinal, irradiation at room temperature (at ~400 nm with 2-mm slits through a monochromator) results in the appearance of fluorescence (at 77°K) and a red shift as well as an increase in the absorption intensity of the longest wavelength band and a decrease of the cis band at ~250 nm. This is clear indication of appreciable cis–trans isomerization to the all-trans isomer.

In summary, the retinals show a marked sensitivity to irreversible destructive photochemistry under high light flux in fluid solutions. Under low light flux or high light flux for short time periods at low temperature (77°K), cis–trans isomerization may occur and a thermally reversible photoproduct is formed. However, ultimately the dominant path appears to be to the reversible photoproduct, particularly with longer irradiation times. At room temperature under low light flux, cis → trans isomerization does occur and appears to be the dominant process (at least for 11-*cis*-retinal).

In view of the fact that at 77°K no bimolecular reactions are possible, the thermally reversible photoproduct(s) must be formed in a unimolecular reaction(s). There appear to be two logical choices for the structure of the photoproduct: I and geometric isomers of II. The absorption spectral maximum of ~300 nm would be most consistent with I. In order to produce this compound from 11-*cis*-retinal, 11–12 and 13–14 double-bond isomerization, 12–13 and 14–15 single-bond isomerization, and ring closure must occur. For the 9-cis and all-trans isomers, the 11–12 double-bond isomerization is not required, and for the 13-*cis*-retinal, no double-bond isomerization is necessary. There is precedence for I since a parallel product has been observed upon irradiation of *trans*-β-ionone.[36] In the case of II, double-bond isomerization is required only for 13-*cis*-retinal. The 14–15 single-bond isomerization and hydrogen atom transfer is required for all the retinals. Generally, the photoenolization reaction of appropriate ketones is considered to proceed *via* a lowest T_{n,π^*} state.[37] It seems quite certain that the lowest triplet state in the retinals is π,π^*. Thus, it would appear that the photoenolization reaction necessary to give II is relatively unlikely. Therefore, we favor structure I as the principal photoproduct despite a rather complex system of photoreactions needed to produce I from 11-*cis*-retinal.



I



II

We shall now discuss the theoretical results and correlate them with the experimental data. Figures 4 and 5 show the potential energy curves for the ground and excited states of 11-*cis*- and 9-*cis*-retinals, respectively, as a function of the angle of twist around the 11–12 and 9–10 double bonds, respectively. The curves were generated employing (a) all singly excited configurations (complete singly excited configurational interaction), (b) the dependence of $\beta_{C=C}$ (eq 1) and $\gamma_{C=C}$ (eq 2) upon the angle of twist, and (c) the contribution of $2p\sigma$ components to the nonadjacent p atomic repulsion integrals (eq 3). As noted in the Theoretical Calculations section, the effect of doubly excited state configurational interaction is known to be important. Therefore we generated the potential energy curves shown in Figure 6 for 11-*cis*- and *all-trans*-retinal. These results include all of the factors noted above plus partial doubly excited state interaction, as noted in the Theoretical Calculations section. Because these curves represent the results of the most thorough theoretical treatment, we shall refer to them in later discussion. We expect that similar curves would exist for any other

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(36) O. L. Chapman, *Advan. Photochem.*, 1, 323 (1969).

(37) K. R. Huffman, M. Loy, and E. F. Ullman, *J. Amer. Chem. Soc.*, 87, 5417 (1965), and references therein.

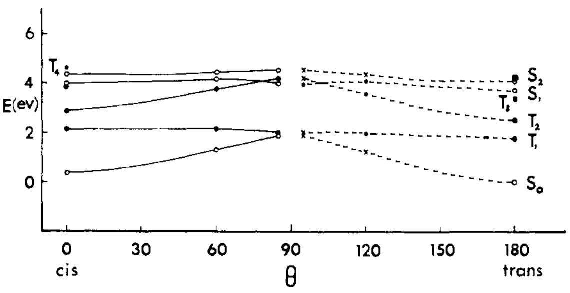

Figure 6. Potential energy curves for twisting around the 11–12 double bond employing eq 1, 2, and 3 and complete singly plus partial doubly excited state configurational interaction. The dashed-line portion is obtained from the solid-line portion based on the energy relationship of curves from 0 to 90° and 90 to 180° of Figure 4: ○, singlet states; and ●, triplet states.

cis isomer and all-trans, based on the similarity of Figures 4 and 5 and other calculations involving each of the cis isomers and the all-trans isomer.

There are several important features to note from Figure 6. The potential barrier to twisting around the double bond in the excited states is considerably less than for the ground state (S_0). The two excited states expected to be of dominant importance in cis ↔ trans photoisomerization are the lowest singlet S_{π,π^*} (S_1) or S_{n,π^*} and the lowest π,π^* triplet (T_1) states. The barrier to isomerization from cis → trans is approximately 0.50 that from trans → cis in S_1 and approximately 0.04 that from trans → cis in T_1. Also, the barrier to cis ↔ trans isomerization in S_1 is higher than that in T_1 and only a very small barrier exists for cis → trans isomerization in T_1; Figure 6. Note the flatness of the T_1 curve compared with all other T_n curves. The T_1 curve is close to degeneracy with the ground state near 90° and T_2 and T_3 cross S_1 at some finite angle. The potential energy curves for the 9-*cis*- and 13-*cis*-retinals are expected to be only slightly different about 90° (see, for example, Figures 4 and 5). The additional consideration of the methyl groups would introduce some slight assymmetry as would the specific inclusion of steric interaction, particularly for the 11-*cis*-retinal.

It is worthwhile noting that these barriers for twisting around a single bond in the ground state are considerably less than that for a double bond.[11] However, the barrier to single-bond twisting in S_1 and T_1 is considerably higher than in the ground state and, moreover, higher than for double-bond twisting (see Figure 4 of ref 11).

We shall now consider the specific significance and relation of the theoretical results, Figure 6, to the experimental results when the S_{π,π^*} is the lowest excited singlet state. Excitation to S_1 could result in cis ↔ trans isomerization, fluorescence, and/or internal conversion the ground state and intersystem crossing to T_1 *via* a T_{π,π^*} state (T_2 or T_3) or T_{n,π^*} followed by emission, cis ↔ trans isomerization, and/or internal conversion to the ground state. For *all-trans*-retinal at 77°K in a rigid matrix and considering the potential barriers, we would expect a moderately intense fluorescence, little internal conversion, some intersystem crossing, and little isomerization, especially to 11-*cis*- and 9-*cis*-retinals (also see later discussion). This is what is found experimentally, except we do not know the value of Φ_{IS} at 77°K. However, at room temperature, Φ_{IS} is estimated to be 0.11.[13] In the case of the 9-cis and 13-cis isomers, based on the potential energy curves, we expect more intersystem crossing and cis → trans isomerization in S_1 or T_1 and therefore a lower Φ_F. This also agrees with experiment. In the case of the 11-cis isomer particularly, including the effect of steric interaction (*vide supra*), more cis → trans isomerization and intersystem crossing is expected with consequent fluorescence quenching.

For 11-*cis*-retinal it is possible the S_{n,π^*} is the lowest excited singlet state (*vide supra*). Consequently, excitation to S_1 would be followed by internal conversion to S_{n,π^*} and subsequently, some internal conversion to S_0, a high degree of intersystem crossing to T_1, and cis → trans isomerization in S_{n,π^*} and T_1. This is in agreement with experiment regarding the fluorescence quenching and isomerization. Φ_{IS} is not known for the 11-cis isomer. On the basis of the barrier heights in the S_1 and T_1 states, we expect cis → trans isomerization will be greater in T_1 for all of the cis isomers.

The above situations are complicated by the fact that upon warming a 3-methylpentane solution of the retinal from 77°K, the Φ_F becomes essentially zero for the 9-*cis*-, 13-*cis*, and *all-trans*-retinals. Thus, the quantum yield of fluorescence has been converted to an equivalent yield of another process(es). On the basis of the barrier heights and small temperature increase, we would not expect that this process would be isomerization for the all-trans isomer. The important aspect of the temperature increase must be the fact that there is a notable decrease in the viscosity. Thus, either or both an increase in internal conversion or intersystem crossing could occur because of freedom to twist. Potentially, both processes could become more probable. That is, the S_1 state would be degenerate with some energy level of T_2, T_3, or T_{n,π^*} at an angle that was attainable, and since the S_0 state energy increases more rapidly with angle than does S_1, the vibronic coupling of S_1 and S_0 would increase. If, indeed, we can expect Φ_{IS} to be 0.11 at 100°K, then most all of the fluorescence quanta would be converted into internal conversion quanta originating in S_1. One must hedge for several reasons. On the basis of 3-methylpentane data as a function of temperature and the 3-methylpentane and glycerol–methanol comparison at 298°K, twisting is known to occur in fluid solution. It is thus possible that intersystem crossing could occur *via* S_{n,π^*} to T_3 or *via* T_3 directly (Figure 6), followed by internal conversion to T_1 with the molecule twisted at a finite angle. From here the molecule could rapidly internally convert to S_0 and also relax in T_1 to an angle near 0°. The number of molecules counted in the triplet–triplet absorption determination would only be those that relaxed in T_1 to near planarity. Thus, the actual Φ_{IS} could be greater than determined in the triplet–triplet absorption procedure.[13] Also, there are the complications of the known wavelength dependence for the fluorescence at 77°K and the isomerization at 298°K (see later discussion), whereas there is an assumed wavelength independence of Φ_{IS} at 298°K. Further, a photoproduct is formed, and if it is of the structure I, then some isomerization (to 13-cis) must occur. However, the latter is expected to be small based on the potential energy barriers (Figure 6). In the case of the cis isomers, a viscosity barrier must also exist at 77°K but apparently it is not sufficiently high to stop isomerization by the mechanism previously described.

At room temperature, the fluorescence quantum yield is essentially zero as at 90°K and other intermediate temperatures. Also, our work as well as that of others clearly indicate that cis ↔ trans isomerization occurs. Although the barriers to isomerization in S_1 are higher compared to those in T_1, some isomerization could occur in S_1. Intersystem crossing can occur to T_1 *via* T_{π,π^*} (T_2 or T_3) or T_{n,π^*}, and twisting can occur because of the low potential barrier; Figure 6. However, the trans → cis twisting should be considerably less likely than cis → trans because of a substantially higher barrier. The inference is that more of the quanta emitted as fluorescence at 77°K are utilized for cis → trans isomerization than for trans → cis at room temperature.

Several other salient features should be noted. It is clear from the potential barriers, Figure 6, that the barrier to trans → cis isomerization is considerably higher than that for cis → trans. That is, there should be a relatively low activation energy for cis → trans isomerization and a definitely higher activation energy for trans → cis isomerization. Thus, cis → trans processes should show only a small temperature dependence. A clear dependence of the quantum yield of fluorescence upon wavelength exists at 77°K. In several cases, this has been shown to be the result of competition between photochemistry and internal conversion at each vibronic level of the S_1 state (as well as higher singlet states).[15,38] Again, unfortunately, at least two kinds of photochemistry can occur, cis ↔ trans isomerization and formation of another photoproduct (as I) (*vide infra*). It is obvious that the likelihood of photochemistry (either or both types) increases as the upper vibronic levels are excited (the wavelength of excitation is shorter). At room temperature, the photoproduct formed at low temperature is thermally reversible (~85%) to the original retinal. Since no monitorable emission exists at room temperature, no wavelength dependence of emission from the retinals can be established. Nonetheless, based on the low-temperature result, it would appear that a wavelength dependence would occur.

After completion of this research, we became aware of work which evaluated the quantum efficiencies of photoisomerization of *all-trans-*, 9-*cis-*, 11-*cis-*, and 13-*cis*-retinals.[39] Furthermore, the temperature and wavelength dependences of the quantum efficiencies were investigated.[39] We wish now to consider these results in light of our conclusions based on the emission and theoretical considerations. The quantum efficiencies (exciting at 365 nm) of cis ↔ trans isomerization reported are 0.4 for 13-cis, 0.5 for 9-cis, 0.2 for 11-cis, and a minimum of 0.06 and maximum of 0.2 for all-trans at 25°.[39] Furthermore, these quantum efficiencies decreased at −65° (208°K) in all cases except for the 11-*cis*-retinal. At 208°K, the values are reported to be 0.1 for 13-cis, 0.25 for 9-cis, 0.6 for 11-cis, and a minimum of 0.002 and a maximum of 0.005 for all-trans. Furthermore, the quantum efficiency of photoisomerization of 11-cis → all-trans varied ~200% depending upon the wavelength of excitation, (~0.09 at ~255, ~0.22 at ~365, and ~0.16 at ~405 nm). The quantum efficiency of all-trans → cis varied up to 300%, depending on the wavelength of excitation and whether maximum or minimum values were used.[39]

The data relative to the dependency of the quantum efficiency of photoisomerization upon temperature are in agreement with our theoretical prediction (*vide supra*), with the exception of the 11-*cis*-retinal. That is, there should be a small activation energy to photoisomerization for the 9-*cis-* and 13-*cis*-retinals and a higher one for *all-trans*-retinal and, therefore, a notable temperature dependence for the isomerization of *all-trans*-retinal. The data on 11-cis[39] is strange, and we see no obvious theoretical or experimental argument at present that would explain the increase in quantum efficiency of photoisomerization with a decrease in temperature.

The fluorescence of all of the retinals is quenched when the temperature is raised from 77°K (rigid) to ~95°K (viscous solution). We argued that particularly on the basis of the data on the all-trans isomer (both experimental and theoretical), the small temperature increase would not result in the conversion of the quantum yield of fluorescence into an equivalent quantum yield of photoisomerization. That is, the principal source of fluorescence quenching arose ultimately from internal conversion. This interpretation is supported by the fact that the maximum quantum yield of photoisomerization of all-trans at 208°K is 0.005. With a further decrease in temperature to ~95°K, it is obvious that the quantum yield of photoisomerization would be small and internal conversion must dominate. Although the decrease in the quantum yield of photoisomerization with a decrease in temperature for the 9-*cis-* and 13-*cis*-retinals is not so marked, there is, nonetheless, a decrease. Therefore, again, an increase in the probability of internal conversion would at least in part be responsible for quenching of fluorescence of these two cis isomers.

Our original calculation of the potential energy curves for twisting around the 11–12 double bond[7] also clearly indicated that the photoisomerization of 11-cis → all-trans should have a noticeably smaller barrier (activation energy) than the all-trans → 11-cis process. Later calculations[8,10] confirmed this prediction. The present calculations represent an extension to the other retinals and a considerable increase in the sophistication of calculations, particularly those appropriate to Figure 6. The most recent calculations[10] show that considerable barriers to photoisomerization exist in both the lowest singlet and triplet states, although it is lowest for 11-*cis*-retinal (to *all-trans-*) in the triplet state.

Triplet–triplet absorption has been observed for the *all-trans*-retinal at 450 nm.[13] Theoretical calculations of the triplet-state energies will vary with the approximation used. For example, we have obtained values of 459 ($T_1 \rightarrow T_6$), 464 ($T_1 \rightarrow T_6$), 449 ($T_1 \rightarrow T_5$), and 363 nm ($T_1 \rightarrow T_7$) depending on whether a Pariser–Parr or Mataga approximation is used for the repulsion integrals and whether a bond order correction is used or not (and what values are assigned to the parameters within the latter). Thus, such energy calculations are of little assistance in deciding the T_n state to which the transition occurs. However, we have also calculated the oscillator strengths (f numbers) for all $T_1 \rightarrow T_n$ transition, considering the possible approximations as

(38) N. Tyer and R. S. Becker, *J. Amer. Chem. Soc.*, **92**, 1295 (1970).

(39) A. Kropf and R. Hubbard, *Photochem. Photobiol.*, in press.

noted above. With this additional information, the triplet–triplet transition is uniquely assigned as $T_1 \rightarrow T_7$. That is, no matter what approximation is used for the repulsion integrals and whether the bond order correction is included or not (and with different values for parameters within the latter), the $T_1 \rightarrow T_7$ oscillator strength is a minimum of 50 (to several hundred) times greater than that of the next strongest $T_1 \rightarrow T_n$ transition. The best calculation energywise predicts the transition to be a $T_1 \rightarrow T_7$ type at 371 nm with $f = 1.37$. The lowest f number calculated for the $T_1 \rightarrow T_7$ transition is 1.05 (highest is 1.84).

Summary

all-trans-,[40] 9-*cis*-, and 13-*cis*-retinals exhibit a fluorescence at 77°K which is quenched in viscous or fluid solution at all temperatures. The *11-cis*-retinal shows no emission (≤ 0.001) to 8200 Å from 77 to 298°K. The quantum yields of fluorescence of *all-trans*- and 9-*cis*-retinals are dependent upon the wavelengths of excitation. The fluorescence is assigned as originating from the lowest S_{π,π^*} of the retinals. In the case of 11-*cis*-retinal, it is possible that the lowest singlet excited state is $S_{n,\pi}$.

Photoisomerization does occur in the case of 9-, 11-, and 13-*cis*-retinals at 77°K and at higher temperatures. In addition, for all the retinals considered, a photo and thermally reversible photoproduct is produced, at 77°K, which is particularly noticeable after extended irradiation.

Theoretical calculations (SCF–MO–CI) have been carried out to determine the potential energy curves resulting from twisting around certain double bonds. The most sophisticated of these calculations includes a consideration of complete singly excited state configurational interaction, the dependence of resonance and repulsion integrals upon the angle of twist, the contribution of 2pσ components to the nonadjacent p atomic repulsion integrals, and partial doubly excited state configurational interaction. These curves have been related to experimental emission and photoisomerization results. It can be predicted that there should be a notably higher activation energy for trans → cis compared with cis → trans isomerizations. This is in agreement with experiment. The theoretical and experimental data, particularly on *all-trans*-retinal, indicate that the fluorescence quenching noted above largely originates from an increase in internal conversion most likely originating in the lowest excited singlet state.

The potential energy curves (and considering viscosity) are consistent with the emission data obtained for the retinals. Based on the potential energy curves, considerable intersystem crossing is expected and the photoisomerization is expected to occur in the lowest excited singlet state (π,π^* or n,π^*) and the π,π^* triplet states. The lowest π,π^* triplet state has a barrier less than that of the lowest π,π^* singlet state. Additional specific consideration of the methyl groups and steric interactions should somewhat modify the relative symmetry properties of the potential energy curves around 90° for the different isomers. However, this should introduce only subtle features into the considerations made.

The triplet–triplet absorption band experimentally observed for *all-trans*-retinal has been assigned as arising from the $T_1 \rightarrow T_7$ transition.

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(40) It has been reported[5] that a fresh sample of *all-trans*-retinal from a particular source did not show fluorescence. We have examined several different samples of *all-trans*-retinal from the source noted in the Experimental Section and all show the same fluorescence regarding band shape and location (see text). In all cases, the samples were obtained from newly opened glass sealed bottles and examined in both degassed and nondegassed solutions at 77°K. In addition, we examined fresh *all-trans*-retinal from the same source that was reported not to emit[5] and did obtain a fluorescence (at 77°K) that was the same as those noted above and in the text (no quantum yields were determined).