Visual Pigments. 8. Hydrogen Bonding Effects on Fluorescence Properties of Retinals

Takeshi Takemura, P. K. Das, Gordon Hug, and Ralph S. Becker*

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77004. *Received February 21, 1977*

Abstract: Dry *all-trans*- and 13-*cis*-retinals in dry alkane solvents show no fluorescence, $\phi_F \leq 10^{-4}$ at 77 K and $\leq 10^{-5}$ at room temperatures. These retinals when H bonded do show fluorescence at temperatures ranging from 77 K to near room temperature. The excitation wavelength dependence of the apparent fluorescence quantum yield arises because of the coexistence of nonfluorescent free retinal and fluorescent H-bonded retinal. The intrinsic quantum yields are excitation wavelength independent within 25%. The data are consistent with a $^1(n,\pi^*)$ state being the lowest excited singlet state in free retinal and a $^1(\pi,\pi^*)$ state being lowest in H-bonded retinal. The conclusions are supported by data on homologues of retinals having both longer and shorter polyene chains.

Introduction

In recent years the wavelength dependence of the fluorescence quantum yield of retinals in condensed phases has presented a difficulty to those trying to understand state order in these compounds. At first glance this phenomenon seems to violate a generally held belief that before an excited molecule in a condensed medium can undergo photochemistry, fluorescence, or intersystem crossing, it must relax to the lowest excited state that has the same spin multiplicity as the ground state. Although there are exceptions to this belief (for example, see ref 1), it has proved to be valid for most large molecules studied in a variety of condensed environments.

The wavelength dependence of the fluorescence quantum yield, ϕ_F, of the retinals was first reported in *all-trans*-retinal.[2] It was noticed that the fluorescence quantum yield of *all-trans*-retinal in 3-methylpentane at 77 K was the largest when retinal was excited near its absorption onset. At shorter wavelengths the quantum yield decreased significantly. This phenomenon was confirmed by other workers who often used different solvent systems.[3-8]

A variety of explanations have been offered for the dependence of ϕ_F on exciting wavelength such as (a) emission from a $^1(n,\pi^*)$ state,[8] (b) competitive photochemistry in upper vibronic levels or states,[2-4] (c) the presence of dimers,[7] and (d) a competitive radiationless process from a $^1(n,\pi^*)$ lying above a lowest $^1A_g^*$ state.[5] Despite these explanations, several nagging points remained unresolved, and in some cases ad hoc assumptions were required.

Given the difficulties in obtaining a definitive explanation of the wavelength dependence problem, we decided to look more closely at the details of this phenomenon. Since in hydrogen-bonding solvents there are spectral shifts of retinals, and since the extent of the wavelength anomaly is somewhat different in hydrogen-bonding and non-hydrogen-bonding solvents, we felt that a detailed study of the effects of H bonding on the wavelength dependence might prove to be of significant value. In a previous communication[9] we reported the conclusions of this study. There we found that the wavelength dependence was due to the existence of two species, one of which is H bonded and fluorescing and the other which is non-H-bonding and nonfluorescing. In this paper we give the details of the argument for this conclusion. In addition we discuss the implications of this conclusion for the problem of state order considered earlier.[9]

The accompanying paper concerns itself with the separate but related problem of dimers and the consequences of the existence of dimers upon the excitation wavelength dependence of ϕ_F.

Experimental Section

all-trans- and 13-*cis*-retinal obtained from Sigma Chemical Co. were used both without and with further purification by high-pressure liquid chromatography. Both the samples were recognized to be essentially identical regarding the fluorescence emission and excitation spectral properties. The 3-methylpentane (3MeP) was purified by the same procedure as described previously.[4] EPA, ethanol (EtOH), methanol (MeOH), trifluoroethanol (TFE), phenol (PhOH), acetonitrile, and dichloromethane were of reagent grade or higher. The latter two solvents were dried with 3A molecular sieves. None showed fluorescence upon excitation in the region of interest. Especially dried retinal and other solutes as well as solvents were obtained. The solutes were pumped under high vacuum (10^{-5} Torr) for 5 h. Purified 3MeP was treated with metallic sodium or 3A molecular sieves in vacuo and distilled onto the dried solute in an emission cell in vacuo.

Absorption, emission, and excitation spectra were measured with the same apparatus as described previously[4] using a front face configuration for emission studies. The photomultiplier–emission monochromator system was calibrated with a standard tungsten source. Quantum yields were obtained using 9,10-diphenylanthracene as a reference[4] and the exciting lamp–monochromator system output was determined using a calibrated thermopile (Eppley Co.) and an ethylene glycol solution of rhodamine B.

Fluorescence lifetimes were also obtained by using the same apparatus and procedures as described earlier.[10]

Because of the potential presence of two absorbing species we were especially concerned about the inner filter effect and excitation spectra.

In the case where two absorbing species may be in solution, the apparent fluorescence intensity, $dI_{\lambda'}(\lambda)$, monitored at λ' to $\lambda' + d\lambda'$ with excitation at λ, generally can be expressed as in eq 1. We have omitted instrumental constants such as spectral sensitivity of the analyzing system and the spectral irradiance of the source for simplicity.

$$dI_{\lambda'}(\lambda) = \frac{1 - 10^{-A_T(\lambda)}}{A_T(\lambda)}\{A_1(\lambda)F_{1\lambda}(\lambda') + A_2(\lambda)F_{2\lambda}(\lambda')\}\,d\lambda' \quad (1)$$

$A_T(\lambda) = A_1(\lambda) + A_2(\lambda)$ and $A_1(\lambda)$ and $A_2(\lambda)$ are the absorbances of the species 1 and 2 at λ, respectively. $F_{1\lambda}(\lambda')$ and $F_{2\lambda}(\lambda')$ represent the fluorescence spectra with the excitation at λ for species 1 and 2. The apparent fluorescence spectrum $F_\lambda(\lambda')$ with the excitation at λ is represented as

$$F_\lambda(\lambda') = A_1(\lambda)F_{1\lambda}(\lambda') + A_2(\lambda)F_{2\lambda}(\lambda') \quad (2)$$

Assuming that the shape of the fluorescence spectrum for each of the two compounds being considered is independent of the excitation wavelength in a condensed phase, it is convenient to write

$$F_\lambda(\lambda') = \phi_1(\lambda)f_1(\lambda'),\ F_{2\lambda}(\lambda') = \phi_2(\lambda)f_2(\lambda') \quad (3)$$

$$\int_0^\infty f_1(\lambda')\,d\lambda' = \int_0^\infty f_2(\lambda')\,d\lambda' = 1$$

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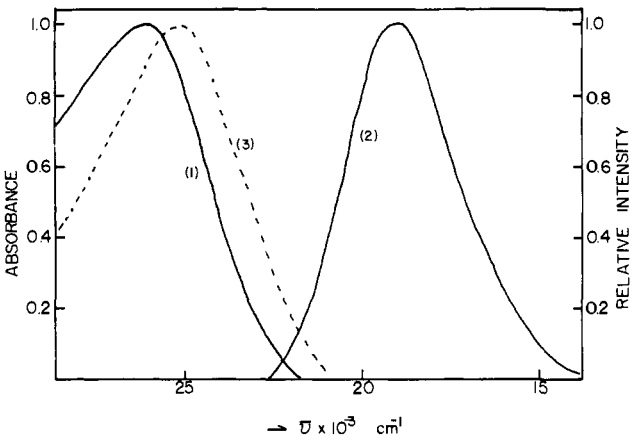


Figure 1. Absorption (1), fluorescence (2), and fluorescence excitation spectra (3) of ATR in undried 3MeP at 77 K.

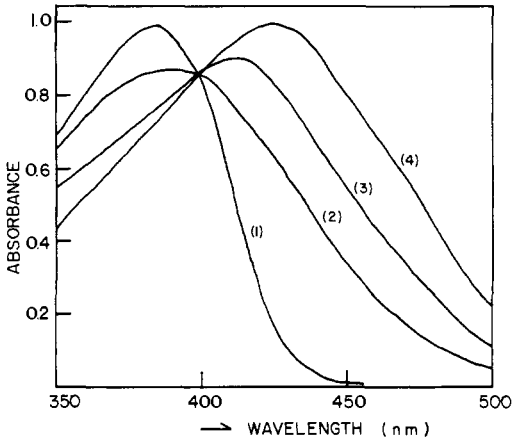


Figure 2. Absorption spectra of ATR-PhOH in 3MeP at 77 K: (1) 0; (2) 1.0×10^{-4}; (3) 2.5×10^{-4}; (4) 1.0×10^{-3} PhOH.

where $\phi_1(\lambda)$ and $\phi_2(\lambda)$ are the intrinsic fluorescence quantum yields with the excitation at λ for the species 1 and 2, respectively, and $f(\lambda')$ is the intrinsic fluorescence spectrum. Therefore, the apparent fluorescence yield, ϕ_{app}, is written as follows:

$$\phi_{app}(\lambda) = \frac{\int dI_{\lambda'}(\lambda)}{1 - 10^{-A_T(\lambda)}} = \frac{I(\lambda)}{1 - 10^{A_T(\lambda)}} = \frac{A_1(\lambda)}{A_T(\lambda)}\phi_1(\lambda) + \frac{A_2(\lambda)}{A_T(\lambda)}\phi_2(\lambda) \quad (4)$$

In general, when $A_1(\lambda)/A_2(\lambda)$ is not a constant for all λ, the apparent fluorescence spectrum (eq 2) will change shape as the exciting wavelength, λ, changes.

In order to make a correspondence between the excitation spectrum and the absorption spectrum, the excitation spectrum $E_{\lambda'}(\lambda)$ monitored at λ' can be defined as

$$E_{\lambda'}(\lambda) = \frac{dI_{\lambda'}(\lambda)}{1 - 10^{-A_T(\lambda)}} A_T(\lambda) = F_\lambda(\lambda')\, d\lambda' = A_1(\lambda)\phi_1(\lambda)f_1(\lambda') + A_2(\lambda)\phi_2(\lambda)f_2(\lambda') \quad (5)$$

Here we are merely converting the measured excitation spectrum that would correspond to a percent absorption spectrum to an excitation spectrum that can be related to the absorption spectrum in terms of absorbance (optical density). The excitation spectrum (eq 5) will change shape as the monitoring wavelength, λ', is changed if $f_1(\lambda')$ and $f_2(\lambda')$ have different shapes and if both are nonzero. Thus in the case that we will be concerned with in this paper where the fluorescence spectrum does not change with exciting wavelength, and where the excitation spectrum does not change with monitoring wavelength, either (a) A_1/A_2 is not constant and only one species fluoresces or (b) A_1/A_2 is constant and $f_1(\lambda') = f_2(\lambda')$. For case (a) $\phi_{1\lambda} = 0$ and

$$E_{\lambda'}(\lambda) = A_2(\lambda)\phi_2(\lambda)f_2(\lambda') \quad (6)$$

$$\phi_{app}(\lambda) = \frac{A_2(\lambda)}{A_T(\lambda)}\phi_2(\lambda) \quad (7)$$

From eq 6 the absorption spectrum of the emitting species can be obtained. $E_{\lambda'}(\lambda)$ is really an excitation spectrum that is corrected by the amount of light absorbed by the nonemitting species in a mixture containing both an emitting species and a nonemitting species. This phenomenon is commonly called the "inner filter effect". In case (b) species 1 and 2 have both identical absorption and identical emission spectra. Of course, eq 1-5 hold in the case of one absorbing and emitting species involved since $A_T(\lambda) = A_2(\lambda)$ and $A_1(\lambda) = 0$.

Equations 1-7 are still valid if species 2 (fluorescent) converts to species 1 (nonfluorescent) but not if species 1 converts to species 2 during the lifetimes of the excited states.

Results

We first give the following evidence. When *all-trans*-retinal (ATR) and 13-*cis*-retinal (13CR) solutions are prepared under dry conditions with dry 3MeP at $1\text{-}2 \times 10^{-5}$ M, no fluorescence is observed ($\phi_{app} \leq 10^{-4}$) at any temperature down to 77 K.[11] Similarly, ϕ_{app} is $\leq 10^{-4}$ for the same retinals in the highly polar but non-hydrogen-bonding solvents, dichloromethane to 183 K and acetonitrile to 230 K (just above the melting point of the solvents). On the other hand, fluorescence is able to be observed in methanol, EPA, and trifluoroethanol and at temperatures up to room temperature.[9,10]

If a very small amount of water (50 μL or less to 1-2 mL of 3MeP to saturate with water) is added to the dried solution of the retinals in 3MeP, fluorescence can be observed at 77 K. The fluorescence spectrum of ATR under the foregoing conditions has a spectral maximum at 520 nm. This fluorescence spectrum is the same as that observed when an ATR solution in 3MeP is not carefully dried. Furthermore, the addition of water to this undried sample enhances the intensity of the fluorescence without any other emission spectral changes.

In Figure 1, the fluorescence and the fluorescence excitation spectra for the undried sample of ATR are shown, together with the absorption spectrum at 77 K. It was observed that the excitation spectra for the undried sample and the samples with water added to the dried and the undried samples were identical after correction for inner filter effects, vide supra.

The results that the excitation and fluorescence spectra do not change in shape and position by the addition of water indicate that only one species is responsible for the fluorescence and that species is the hydrogen-bonded complex between the retinals and water. Unfortunately, we could not make quantitative measurements in these experiments concerning retinals-H_2O-3MeP because of the low solubility of water in 3MeP.

In order to confirm the hydrogen bonding effects on the fluorescence properties of the retinals, we shall present the following experimental results with phenol as a proton donor. Figure 2 shows the change in absorption spectrum of ATR in 3MeP at 77 K caused by the addition of phenol. The spectra are shifted to the red as the concentration of phenol increases and clearly show an isosbestic point. Therefore, this spectral change is considered to be due to the formation of the hydrogen bonded complex between ATR and phenol as:

$$\text{ATR} + \text{PhOH} \overset{K}{\rightleftharpoons} \text{ATR} \cdot \text{PhOH} \quad (8)$$

This is further supported by the spectral change in the absorption spectrum of phenol in the form of addition of shoulders to the long-wavelength side of the vibrational components in the first transition. Using the Benesi-Hildebrand method,[12] and keeping the concentration of phenol sufficiently low such that the fraction of the molecules that are complexed is relatively small and the self-association of phenol is negligible, we obtain $K = 5.8 \times 10^3$ M^{-1} for the equilibrium of eq 8 in 3MeP at 77 K (Figure 3). The wavelength (440 mm) monitored is in a spectral region where only the H-bonded form absorbed.

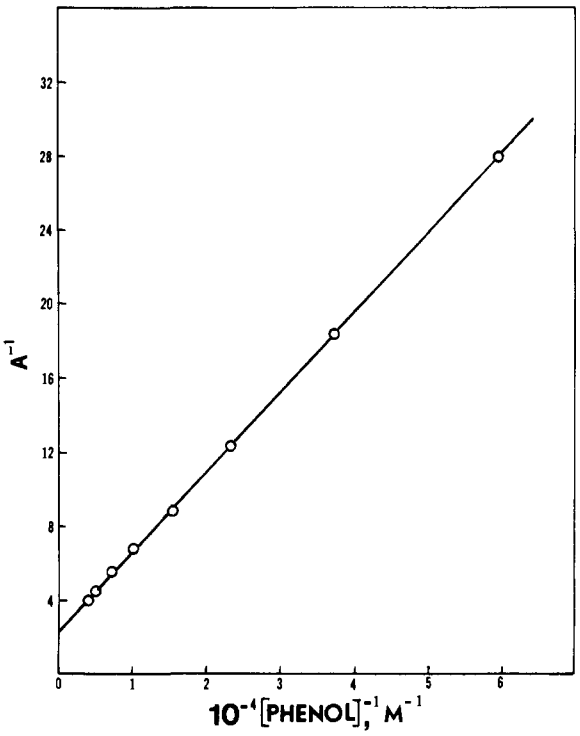


Figure 3. Benesi-Hildebrand plot for ATR-PhOH in 3MeP at 77 K.

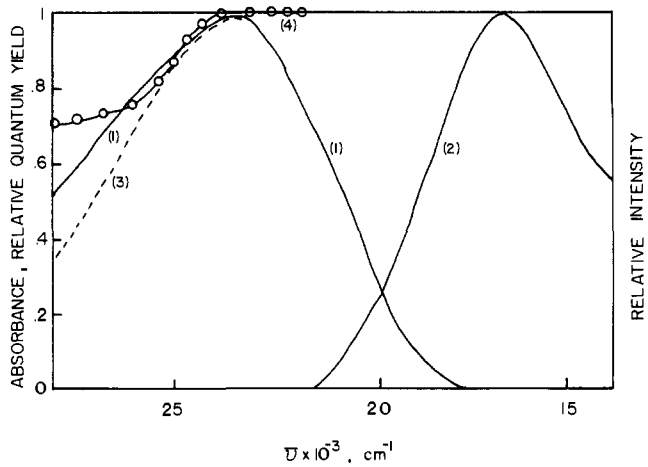


Figure 4. Absorption (1), fluorescence (2), and excitation spectra (3) and relative quantum yield (4) of ATR plus PhOH (10^{-3} M) in 3MeP at 77 K.

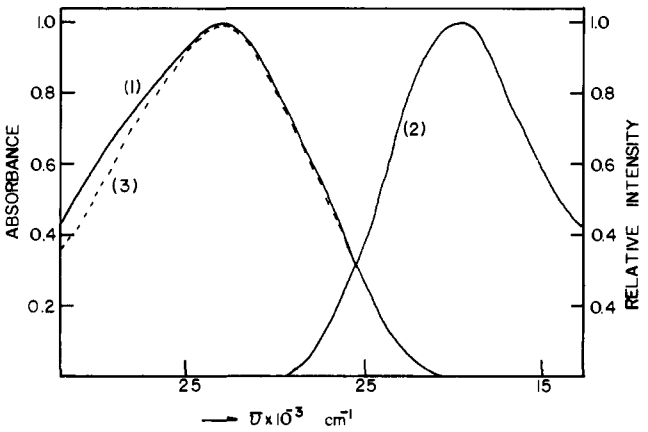


Figure 5. Absorption (1), fluorescence (2), and excitation spectra (3) of ATR-PhOH (3×10^{-2} M) in dichloromethane at 183 K.

Figure 4 shows the absorption, fluorescence, and excitation spectra for ATR plus phenol in 3MeP at 77 K. The apparent fluorescence quantum yields (ϕ_{app}) are also shown as a function of the excitation wavelengths. The results obtained from detailed experiments are as follows: (1) The shape and the position of the fluorescence and the excitation spectra (corrected for inner filter effect) were essentially independent of the phenol concentrations (up to $\sim 2 \times 10^{-3}$ M phenol for $\sim 4 \times 10^{-5}$ M ATR). This indicated that primarily one species was formed under the conditions of our experiment. (2) The apparent quantum yields as a function of the excitation wavelength approached a constant as the phenol concentration increased. (3) At a relatively high concentration of phenol ($\sim 2 \times 10^{-3}$ M for $\sim 4 \times 10^{-3}$ M ATR) where a major fraction of the ATR molecules was expected to be hydrogen bonded, the corrected excitation spectrum nearly coincided with the absorption spectrum showing that the intrinsic quantum yield of the complex was essentially wavelength independent (within 25%).

As expected from the inner filter effect (eq 7) the apparent quantum yield obtained by excitation at wavelengths shorter than 420 nm (in the spectral region where both free and H-bonded ATR absorbed) was found to increase with increasing concentration of phenol. However, with excitation in the region where only the complex absorbed, we noticed an increase of apparent quantum yield with increasing concentration of phenol. In addition, a Benesi-Hildebrand type plot of E^{-1} against $[PhOH]^{-1}$ (based on eq 6) was found to be nonlinear. These results can be explained if we make allowance for the interconversion between the free ATR and its H-bonded form in their excited states. It can be shown that the effect of this interconversion will be more pronounced on the observed emission intensity than on the observed shape of the excitation spectra. An alternative explanation may be in terms of more than one complex being formed between phenol and ATR. More concerning this will appear elsewhere.

A separate experiment was performed in order to establish the fact that the interaction between ATR and PhOH was not one of charge-transfer adduct type, but one primarily involving the proton of phenol. When a small amount of diethyl ether ($\sim$1%, v/v) was added to the 3MeP solution containing retinal (4×10^{-5} M) and PhOH ($\sim 2 \times 10^{-3}$ M), no complex was formed as shown by the complete absence of the long-wavelength absorption and emission. There is, however, possibly a large charge-transfer contribution to the H bonding between PhOH and ATR, resulting in the large red shift in both absorption and emission of the phenol-retinal complex (compared to the cases of H_2O or MeOH complexes).

As noted already, the fluorescence of ATR could not be observed in dichloromethane even at 183 K. However, when small amounts of phenol were added to the dichloromethane solution of ATR, the fluorescence could be easily observed even at 270 K. Figure 5 shows the absorption, fluorescence, and excitation spectra for ATR in dichloromethane including phenol at 3×10^{-2} M at 183 K. Results similar to these concerning the concentration dependence of the fluorescence properties for ATR-PhOH in 3MeP at 77 K were found, vide supra. Thus, the experimental results for ATR-PhOH in dichloromethane under fluid conditions again indicated that the fluorescence was from the hydrogen-bonded species and showed the essential independence of the intrinsic quantum yield of retinal fluorescence upon the excitation wavelength.

From the absorption spectral change of the retinals caused by the addition of phenol to dichloromethane, we could obtain from a Benesi-Hildebrand plot the values $K = 80\ M^{-1}$ at -60 °C and $K = 140\ M^{-1}$ at -90 °C based on the equilibrium of eq 8 ($\Delta H = -1.6$ kcal/mol).

Discussion

In the previous section and elsewhere[9] it was found that

ATR and 13CR show no fluorescence ($\phi_{app} \leq 10^{-4}$) in any aprotic solvents. On the other hand, the addition of a proton donor resulted in fluorescence of the retinals. The evidence is that a H-bonded retinal species is the one that fluoresces while the non-H-bonded retinal does not. Furthermore, the excitation wavelength dependence of ϕ_{app} for the retinals can be attributed to the coexistence of nonfluorescent, non-H-bonded retinals and fluorescent H-bonded retinals. There is other corroborative evidence regarding the formation of H-bonded retinal. In a study of the relative population of the triplet state of *all-trans*-retinal in various solvents,[13] there was good evidence for formation of an H-bonded complex between the retinal and alcohols. Furthermore, an infrared study[13] showed that a hydrogen-bonded complex between ethanol and *all-trans*-retinal existed. The formation constant at room temperature (25 °C) between retinal and methanol in the ground state was 6.5 (our data with phenol in dichloromethane gave 80 M^{-1} at −60 °C).

We shall now examine the basis for the lack of fluorescence, $\phi_F < 10^{-4}$ for free retinals and the presence of fluorescence for H-bonded retinals. The absence of fluorescence in free retinals cannot be attributed to some intrinsic property associated with the long polyene chain (e.g., large Franck–Condon factors with resultant, relatively large internal conversion rate constant). This is evident from the emission behavior of various polyenes of comparable chain length. Thus, (a) a C_{22} alcohol (having the same number of double bonds as retinal),[14] C_{22} and C_{24} aldehydes (homologues of retinal),[14] and various retinols[8,15,16] fluoresce in various solvents at 77 K and at any temperature (up to 298 K), and (b) retinoic acids,[8] esters,[8] and retinal Schiff bases[4,10] show fluorescence at 77 K. The fluorescence quantum yields of many of these compounds are known to show no, or essentially no, excitation wavelength dependence.[4,10,14,16]

Some insight into the reasons for the difference in the flourescence behavior of free retinal and H-bonded retinals can be gained by considering the available rate constants for photophysical processes in these two species. In the discussion that follows, we shall use the usual kinetic model with three possible independent paths:

$$k = k_r + k_{isc} + k_{ic} = k_r + k_{nr}$$

where k_r is the radiative rate constant, k_{ic} is the internal conversion rate constant, and k_{isc} is the intersystem crossing rate constant. Using the data presented in Table I, we obtain for the HB of ATR in 3MeP at 77 K $k_r \sim 5 \times 10^7\ s^{-1}$, $k_{nr} \sim 10^9\ s^{-1}$, and hence, both k_{ic} and k_{isc} separately $< 10^9\ s^{-1}$. Making the assumption that the fluorescing state in the free ATR is the same as in the HB so that k_r (free) = k_r (HB) $\simeq 5 \times 10^7$, and using the value of $\phi_F < 10^{-4}$ for free ATR in 3MeP at 77 K, we obtain for free ATR:

$$10^{-4} > \phi_F = (k_r + k_{nr})^{-1} k_r \sim (k_r + k_{nr})^{-1} 5 \times 10^7$$

and

$$k_r + k_{nr} > 5 \times 10^{11}\ s^{-1}$$

Ignoring the relatively small magnitude of $k_r \sim 5 \times 10^7\ s^{-1}$, we get $k_{nr} = k_{ic} + k_{isc} > 5 \times 10^{11}\ s^{-1}$ for free ATR. This value appears to be unrealistically large from experience with other molecules. For H-bonded ATR and for many of the previously mentioned polyene systems for which lifetime data are available, $k_{ic} < 10^9\ s^{-1}$ at 77 K. Except for some (possibly) small effects of pseudo-Jahn–Teller distortions, there is no special reason for k_{ic} of free ATR to be much higher than that of the analogous polyenes. As for the k_{isc} part, Hochstrasser et al.[17] measured $k_{isc} = 2.9 \times 10^{10}\ s^{-1}$ for ATR in hexane at room temperature. At the lower temperature (77 K) considered here, k_{isc} would probably be even smaller owing to lack of collision-induced intersystem crossing at lower temperatures (other factors remaining the same).

Table I. Fluorescence Properties of H-Bonded Complexes between ATR and Proton Donors in 3MeP at 77 K

Proton donor	ϕ_F	τ_{obsd}, ns	τ_0,[d] ns
H_2O	0.05 ± 0.01[a,c]	1.0 ± 0.2	20 ± 10
PhOH	0.07 ± 0.01[b,c]	1.6 ± 0.2	23 ± 10

[a] This value was obtained using a sample with water added, and with excitation in the region 440–450 nm. [b] This value was obtained in the presence of $\sim 10^{-3}$ M phenol (for $\sim 4 \times 10^{-5}$ M ATR) with excitation in the region (450 nm) where essentially only the complex absorbed. [c] The true quantum yields are probably higher than the values given here because of the mixed absorption and a possible quenching mechanism involving the interconversion of the excited states of free and complexed ATR (see the text). [d] τ_0 is the natural radiative lifetime.

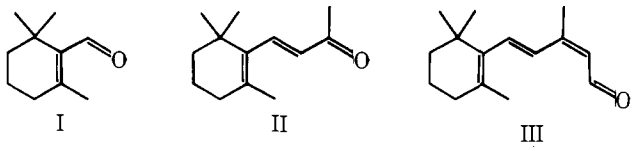
From the foregoing analysis of various rate constants, it appears that the assumption k_r (HB) = k_r (free) is *incorrect*. Information about the lifetime of the lowest singlet state in free ATR can be obtained by assuming a reasonable upper limit for k_{nr}. With $k_{isc} \approx k_{nr} < 10^{10}\ s^{-1}$ (assumed) and $\phi_f < 10^{-4}$ (observed), we obtain for free ATR $k_r < 10^6\ s^{-1}$, or a natural radiative lifetime of >1 μs indicating a weakly allowed state as the lowest singlet state. Thus, one factor in the dramatic change in the fluorescence properties of ATR on H bonding is k_r (free) is considerably less than k_r (HB). As a corollary, if k_{ic} remains the same in free and H-bonded ATR, then these two factors could account for the observed fluorescence behavior. In view of the difficulty in assuming a lower limit for k_r, we can neither definitely establish nor rule out the possibility that k_{nr} (free) > k_{nr} (HB). Nonetheless, it should be noted that a significant decrease in ϕ_{isc} of ATR at room temperature does occur on changing solvent from cyclohexane to methanol[18] or on addition of phenol in hexane.[19] If k_{isc} remains constant, this would indicate a decrease in k_{isc} in the presence of H bonding.

We shall now discuss the possible nature of the weakly allowed, lowest singlet state in free retinals. Of the low-lying $^1(\pi,\pi^*)$ states in polyenes, the one that is weakly allowed is the state loosely called A_g^* state. With this $^1(\pi,\pi^*)$ state lowest and a $^3(n,\pi^*)$ state lying below it in free retinals, a relatively large k_{isc} could be explained in terms of the process $^1(\pi,\pi^*) \rightsquigarrow {}^3(n,\pi^*)$. Hydrogen bonding could then result in an increase in k_r through increased mixing or reversal of position of the $^1A_g^*$ with ^1B_u state and, possibly, a decrease in k_{isc} through raising of the $^3(n,\pi^*)$ state above the $^1A_g^*$ state. However, the estimated natural radiative lifetime of >1 μs for free ATR is longer than most of the natural lifetimes reported until now for those systems in which the $^1A_g^*$ state is believed to be the lowest. For example, in the cases of diphenyloctatetraene and diphenylhexatriene, the radiative lifetimes at room temperature are 49 and 32 ns, respectively.[20] Of all the molecules studied[15,21] only the retinols at room temperature have lifetimes that are close to 1 μs,[15] but most of these are somewhat shorter than the 1 μs lower bound for the lifetime of free retinal. In addition, if we extrapolate from the retinol case, and if there were to be a difference in lifetime between the $^1A_g^*$ states of retinol and retinal, we would expect the lifetime of retinal to be the shorter, because the oxygen involved in the conjugation of retinal would relax the symmetry forbiddenness.

Another reason why a $^1A_g^*$ state appears to be an unlikely assignment for the lowest singlet state in retinal is that it is inconsistent with the data on C_{22} aldehyde. C_{22} aldehyde ($\phi_F \sim 0.1$ at 77 K) has natural radiative lifetimes of 11 and 9 ns at 77 K in 3MeP and EPA, respectively.[14] Experiments on a polyene series[22] indicate that the vibrationless level of the $^1A_g^*$

state decreases faster in energy than the vibrationless level of the ^1B_u state as the polyene chain gets longer. This implies that if a $^1A_g^*$ state is lowest in ATR, it is also lowest in the higher homologue, C_{22} aldehyde, and that ΔE ($^1B_u^* - {}^1A_g^*$) would be larger in C_{22} aldehyde. Consequently, based on a simple intensity borrowing mechanism, C_{22} aldehyde should have a longer natural radiative lifetime than retinal. However, this is contrary to the observed, relatively short radiative lifetime of C_{22} aldehyde in comparison to a long radiative lifetime estimated for ATR. Thus trying to extrapolate an assignment of a $^1A_g^*$ state as lowest in ATR to C_{22} aldehyde leads to another incongruity.

The other state with very low oscillator strength that is a likely candidate for the lowest state in free retinal is a state principally of $^1(n,\pi^*)$ character. The $^1(n,\pi^*)$ assignment fits by analogy to the state order seen in the homologous series. In the case of shorter homologues, β-cyclocitral (I), β-ionone (II), and 9-*cis* C_{15} polyene aldehyde (III), where a singlet $\pi^* \leftarrow n$



transition can be seen in absorption to be of lowest energy, no fluorescence ($\phi_F < 10^{-4}$) is observed.[14] For β-cyclocitral the entire $\pi^* \leftarrow n$ transition can be seen and the oscillator strength in 3MeP at 77 K is 1.7×10^{-3} (corresponding to a lifetime of ~ 1 μs). Only part of the $\pi^* \leftarrow n$ transition can be seen for II and III, and both, when hydrogen bonded with trichloroacetic acid in 3MeP at 77 K, do exhibit fluorescence.[14] Also, aromatic carbonyl compounds with a $^1(n,\pi^*)$ state lowest show no fluorescence,[23,24] $\phi_F < 10^{-4}$. For such a state order in free retinal, reversal of the energy levels $^1(n,\pi^*)$ and $^1(\pi,\pi^*)$ on H-bond formation and possible, essential lack of contribution of the $^1(n,\pi^*) \rightsquigarrow {}^3(\pi,\pi^*)$ pathway to intersystem crossing in the H-bonded form can explain the observed emission behaviors.

Thus, for free ATR, the assignment of a state principally of $^1(n,\pi^*)$ character as lowest appears to be more tenable than that of a state principally of $^1A_g^*$ character. However, this conclusion is not unequivocal because the models we have used for comparing the two assignments are relatively crude for quantitative purposes. In view of the closeness of the three low-lying states, $^1A_g^*$, ^1B_u, and $^1(n,\pi^*)$, the lowest singlet state in free retinal may be of a composite nature with a large amount of $^1(n,\pi^*)$ and $^1A_g^*$ character. H-Bonding may then result in a decrease of $^1(n,\pi^*)$ character and an increase of ^1B_u character. The resultant increased k_r, coupled with a possible decrease in k_{isc}, could explain the observed emission behavior of free and H-bonded retinals.

Conclusions

The wavelength dependence in retinal is due to the existence of two species—one hydrogen bonded and fluorescing and the other non-H-bonded and nonfluorescing. The hydrogen-bond formation either causes a singlet state order change in ATR or a strong solvent-induced mixing in the lowest singlet state. The lowest singlet state in free retinal remains somewhat uncertain, but the evidence points most strongly to a state of principally $^1(n,\pi^*)$ character.

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References and Notes

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