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Visual Pigments. 11. Spectroscopy and Photophysics of Retinoic Acids and *all-trans*-Methyl Retinoate

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Abstract: The photophysics of hydrogen-bonded complexes of retinoic acid and its 9-cis and 13-cis isomers and the photophysics of the dimers of these isomers of retinoic acid were studied. The investigation indicated that complexes of retinoic acid and molecules that form hydrogen bonds with the carbonyl oxygen of retinoic acid (type I complexes) have both higher radiative and nonradiative rate constants than do hydrogen-bonded complexes of retinoic acid and molecules that form hydrogen bonds only with the hydroxyl oxygen of retinoic acid (type II complexes). For *all-trans*-retinoic acid in 3-methylpentane at 77 K, the type I complexes have radiative rate constants approximately equal to or greater than 2×10^8 s^{-1} and nonradiative rate constants greater than 3×10^8 s^{-1}. Both the radiative and nonradiative rate constants of the type II complexes of *all-trans*-retinoic acid at 77 K in 3-methylpentane are less than 1×10^8 s^{-1}. The dimer of retinoic acid (K(association) = 1×10^4 M^{-1} at room temperature for the all-trans isomer) behaves like a type I complex, and its excited-state properties are better understood in terms of hydrogen bonding than in terms of an exciton model. The photophysical properties and triplet-triplet absorption spectrum of methyl retinoate were measured. The study concluded with an examination of some of the implications of this work for the role of hydrogen bonding in the dimers and monomers of retinal and retinol.

Introduction

In recent years, there have been many theoretical and experimental studies[2-10] of the excited state of the retinyl polyenes including retinols, retinals, and retinyl Schiff bases. These studies have been motivated primarily because of the relationship these systems bear to the process of vision. Retinoic acids have so far attracted the least attention of the investigators. The absorption and fluorescence spectra of *all-trans*-retinoic acid in EPA and hydrocarbon solvents have been reported by Thomson.[9] The spectra in that work[9] were apparently considered as being due to the monomeric form of the acid.

In continuation of our interest in the model visual pigments, we undertook the spectroscopic investigation of retinoic acids and *all-trans*-methyl retinoate, henceforth called retinoyl systems when used collectively. This study also became pertinent for comparative purposes when it was observed[4,10] that aggregation (dimer formation) plays an important role in determining the photodynamical behavior of retinals and retinols under certain conditions. Moreover, recent findings regarding the potential of retinoic acids as anticancer agents[11] and the presence of retinoyl complexes as the autofluorescence component of the storage material in neurons from Batten disease[12] establish these compounds as important biomolecules.

It is generally recognized that in retinyl polyenes there are

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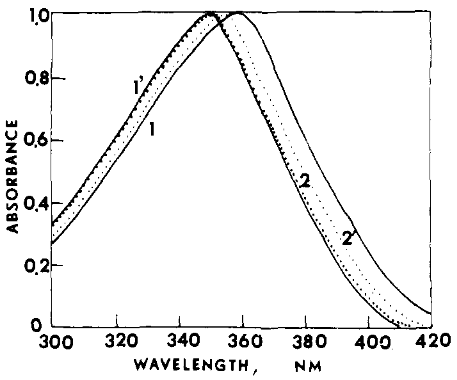


Figure 1. Absorption spectra of *all-trans*-retinoic acid (1 and 1′, solid lines) and *all-trans*-methyl retinoate (2 and 2′, dotted lines) in 3MP (*1* and *2*) and 3MP + 10% ether (1′ and 2′) at room temperature.

three low-lying singlet excited states, ^1B_u, ^1A_g, and $^1(n,\pi^*)$, which play important roles in determining their photodynamical properties. The relative location of these states depends not only on structural factors, such as substitution and geometric distortion in the polyene chain and the nature of the heteroatom at the end of the chain, but also on the environmental conditions including solvents, temperature, and the presence of hydrogen-bonding agents. A state of $^1(\pi,\pi^*)$ character has been assigned as the lowest excited singlet state in retinals in hydrogen-bonding polar solvents,[3] retinols,[5b,10] and retinyl Schiff bases.[2a,13] For some of these systems the state has been shown to be of $^1A_g{}^*$ character on the basis of two-photon excitation spectral studies,[7,14,15] solvent effects,[10] fluorescence lifetime considerations,[1,10] and semiempirical calculations.[6,7] On the other hand, a state of primarily $^1(n,\pi^*)$ character has been assigned as the lowest singlet state in the case of dry retinals in dry hydrocarbon solvents.[3]

Experimental Section

all-trans-Retinoic acid was purchased from Sigma and crystallized from acetonitrile before use. 13-*cis*- and 9-*cis*-retinoic acids were obtained as gifts from Hoffmann-La Roche. They were crystallized from acetonitrile and methanol, respectively. *all-trans*-Methyl retinoate was prepared[16] by refluxing a solution of *all-trans*-retinoic acid and methyl iodide in methyl ethyl ketone in the presence of anhydrous potassium carbonate. The crude ester was chromatographed on a silica gel column using 3% ether in petroleum ether, and then crystallized from *n*-hexane. The solvents/reagents were of spectral grade or were purified by methods described elsewhere.[13,17]

The details regarding the setup and procedures for obtaining the absorption-emission spectral data, fluorescence quantum yields, and nanosecond lifetimes have been given in the previous papers.[2a,17-19] The triplet-triplet spectra and other data concerning triplet states were obtained using the techniques of pulse radiolysis (4-MeV electron pulses, 30-100 ns) and laser flash photolysis (337.1-nm N_2 laser, 9 ns, 3-5 mJ), described in detail in a previous paper.[20] The IR spectra were recorded in a Perkin-Elmer 457 grating spectrophotometer.

Results

Figure 1 shows the absorption spectra of *all-trans*-retinoic acid and its methyl ester in 3-methylpentane (3MP) and in 3MP + 10% ether at room temperature. The absorption spectral maximum of retinoic acid is blue shifted upon going from 3MP to 3MP + ether solvent (or EPA), while that of the ester is slightly red shifted. These effects are more pronounced at 77 K. This spectral behavior of *all-trans*-retinoic acid and its ester can be best explained in terms of self-association of retinoic acid to form a dimer in 3MP and its existence as monomer, hydrogen bonded to alcohol or ether, in EPA or 3MP + ether (see Discussion). An analysis of the concentration dependence of the absorption spectra of *all-trans*-retinoic acid in 3MP at room temperature has been done in a manner similar to that used for benzoic acid.[21] The equilibrium constant for dimer formation

$$2A \rightleftarrows A_2 \qquad A = all\text{-}trans\text{-retinoic acid} \qquad (1)$$

is estimated at 1.0×10^4 M^{-1} (298 K, 3MP).

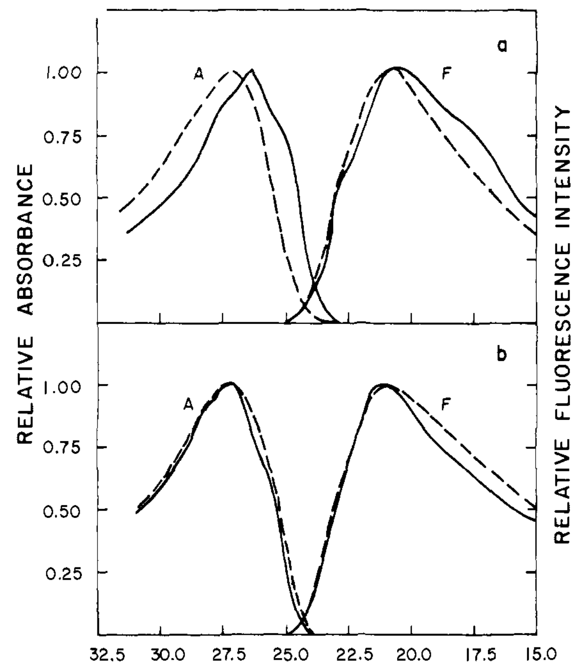


Figure 2. Absorption (A) and fluorescence (F) spectra of *all-trans*-retinoic acid (a) and *all-trans*-methyl retinoate (b) in 3MP (solid line) and 3MP + 10% ether (broken line) at 77 K.

Additional evidence for the existence of hydrogen-bonded dimers and hydrogen-bonded complexes of retinoic acid and ether was obtained from room temperature infrared spectroscopy, using 10^{-2} M solutions of retinoic acid in CCl_4 and CCl_4 plus 10% ether. Both these solutions show very intense, broad peaks near 3000 cm^{-1} in the O-H stretching region that are strongly red shifted from the free O-H stretch (3550 cm^{-1}), indicating that very strong hydrogen bonds exist. The carbonyl stretching region also has evidence of hydrogen-bond formation. The peak near 1680 cm^{-1} is assigned to the dimer and is red shifted relative to the 1713 cm^{-1} of the retinoic acid-ether complex. Both peaks are red shifted relative to the peak at 1725 cm^{-1} due to the monomer which is non-hydrogen-bonded. These assignments are based on (1) study of the concentration dependence of the IR spectra, (2) a detailed comparison between the IR spectra of retinoic acid and methyl retinoate, and (3) a general comparison between the IR spectra of these compounds with similar spectra for other carboxylic acids and esters.[22]

In Figure 2, absorption and fluorescence spectra of *all-trans*-retinoic acid and its ester in 3MP and 3MP + 10% ether at 77 K are shown. None of the spectra changes shape or position over concentrations ranging from 5×10^{-6} to 10^{-4} M. The spectrum of *all-trans*-retinoic acid in 3MP at 77 K is attributed primarily to its dimer while those of *all-trans*-retinoic acid in EPA and 3MP + ether and of *all-trans*-methyl retinoate in 3MP, 3MP + ether, and EPA are due to the respective monomers, including hydrogen-bonded species where applicable (see Discussion). It should be noted that slight structure is observed in both the absorption and emission spectra of *all-trans*-retinoic acid in 3MP at 77 K, and this structure is lost on going to 3MP + ether or EPA.

The absorption-emission spectral behaviors of the cis retinoic acids are similar to that of *all-trans*-retinoic acid. Figure 3 shows their absorption and emission spectra in 3MP and 3MP + 10% ether at 77 K. It is interesting to note that the

Table I. Spectral and Photophysical Data for Retinoic Acids and *all-trans*-Methyl Retinoate at 77 K

compd	solvent	absorption max,[a] nm	emission max,[b] nm	quantum yield[c]	obsd lifetime,[d] ns	natural radiative lifetime, ns	radiative rate constant, $s^{-1} \times 10^{-8}$	nonradiative rate constant, $s^{-1} \times 10^{-8}$
all-trans-retinoic acid	3MP[f]	381	470	0.44[e]	1.7[e]	3.9	2.59	3.29
	3MP + 10% ether	364	468	0.54[e]	8.0[e]	14.8	0.68	0.58
	3MP + 0.1% acetic acid	375	485	0.31	1.6	5.2	1.94	4.31
	3MP + 0.1% TCA[g]	390	550	0.16	0.6	3.8	2.67	14.00
	3MP + 2% triethylamine	319	480	0.25	9.8	39.2	0.26	0.77
	EPA[h]	365	468	0.48	7.5	15.6	0.64	0.69
	2-MeTHF[i]	366	465	0.54	7.0	13.0	0.77	0.66
all-trans-methyl retinoate	3MP	368	468	0.61[e]	7.6[e]	12.5	0.80	0.51
	3MP + 10% ether	368	468	0.62[e]	5.6[e]	9.0	1.11	0.68
	3MP + 0.1 acetic acid	368	468	0.53	6.5	12.3	0.82	0.72
	3MP + 0.1% TCA	382	518	0.22	1.5	6.8	1.47	5.2
9-*cis*-retinoic acid	3MP	373.5	550	0.22	1.3	5.9	1.69	6.0
	3MP + 10% ether	356	542	0.47	4.4	9.4	1.07	1.21
13-*cis*-retinoic acid	3MP	383.5	475	0.45	1.5	3.3	3.00	3.67
	3MP + 10% ether	366	472	0.53	6.3	11.9	0.84	0.75

[a] ±0.5 nm. [b] ±3nm. [c] ± 5 to ±15% in the range 0.6–0.1. [d] ±5 to ±20% in the range 10–1 ns. [e] See ref 25. [f] 3MP = 3-methylpentane. [g] TCA = trichloracetic acid. [h] EPA = ether–isopentane–ethyl alcohol in the ratio 5:5:2 (v/v). [i] 2-MeTHF = 2-methyltetrahydrofuran.

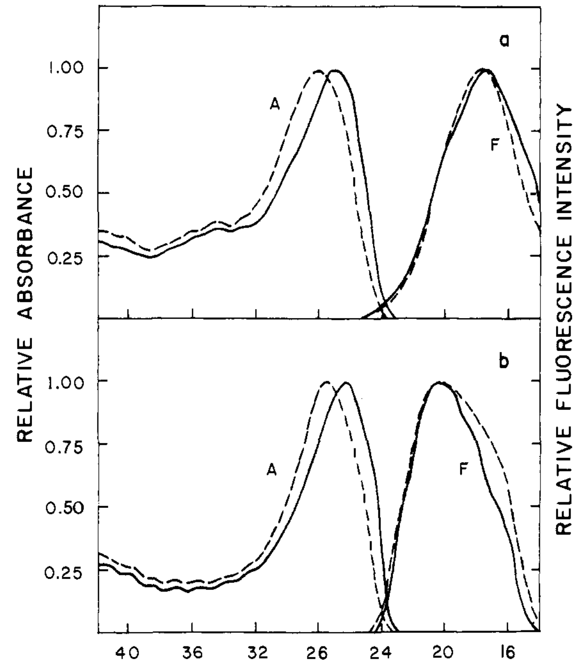


Figure 3. Absorption (A) and fluorescence (F) spectra of 9-*cis*-retinoic acid (a) and 13-*cis*-retinoic acid (b) in 3MP (solid line) and 3MP + 10% ether (broken line) at 77 K.

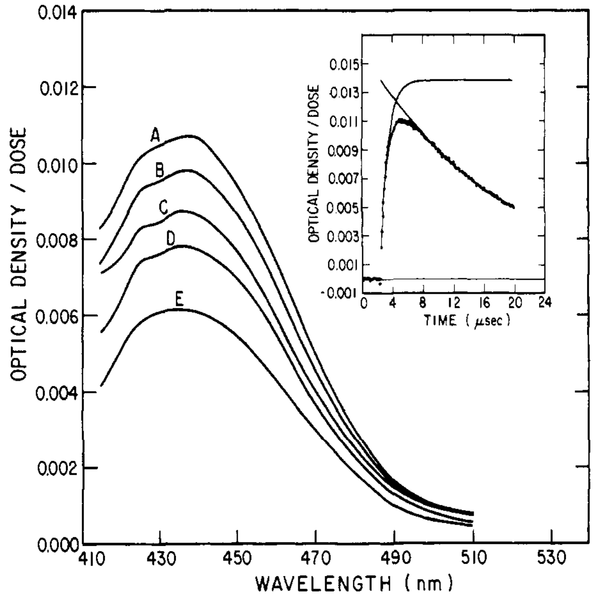


Figure 4. Triplet-triplet spectra of *all-trans*-methyl retinoate in benzene at room temperature, as obtained by energy transfer from biphenyl method of pulse radiolysis. The curves A, B, C, D, and E correspond to the spectra of the transient at times 2.7, 5.4, 1.0, 9.45, and 13.45 μs, respectively. Insert: time profile (buildup followed by decay) of absorbance due to *all-trans*-methyl retinoate triplet at 435 nm.

emission maxima of 9-*cis*-retinoic acid in both 3MP and 3MP + 10% ether at 77 K are located at considerably lower energies than those of the all-trans and 13-cis isomers in similar solvents. This characteristic low-energy location of the emission maxima of 9-cis isomer relative to those of 13-cis and all-trans isomers has also been observed in the case of retinyl Schiff bases[13] and retinals[13] (later identified as hydrogen-bonded complexes).[3]

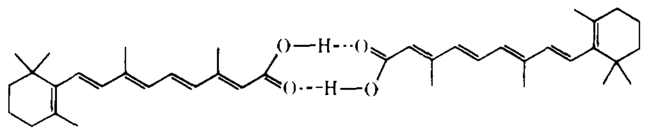
Table I summarizes the absorption-emission spectral data, quantum yields (ϕ_F), and lifetimes of the three retinoic acids and *all-trans*-methyl retinoate under various conditions at 77 K. The quantum yields of the acids (monomeric or dimeric) as well as the all-trans ester are relatively high at 77 K and, in fact, higher than the quantum yields of other retinyl polyenes (e.g., aldehydes,[13] alcohols,[10] and Schiff bases[13]) under similar conditions. There is no dependence of the fluorescence quantum yields upon excitation wavelengths. We could not observe any emission (ϕ_F, $\phi_P < 10^{-4}$) at room temperature in any solvent used for any of the molecules.

The triplet-triplet absorption spectrum (λ_{max} 435 nm, ϵ_{max} 89 000 M^{-1} cm^{-1}) of *all-trans*-methyl retinoate in benzene at room temperature is shown in Figure 4. This has been obtained by the method of pulse radiolysis by energy transfer from biphenyl, the observed rate constant for energy transfer being 8.4×10^9 M^{-1} s^{-1}. With 337.1-nm laser excitation of *all-trans*-methyl retinoate, only a very weak signal is observed in the region of maximum triplet-triplet absorption. The quantum efficiency of the occupation of the lowest triplet state (ϕ_{T_1}) is therefore estimated to be ≤ 0.003. The triplet decay rate constant for *all-trans*-methyl retinoate in benzene is found to be 5.8×10^4 s^{-1}. Practically no signal due to triplet-triplet absorption in the relevant spectral region could be observed for *all-trans*-retinoic acid by direct laser excitation in cyclohexane, benzene, or benzene + 10% ether at room temperature. Using ϕ_{T_1} as a measure of intersystem crossing efficiency (see Discussion) we conclude that practically all of the quanta absorbed by *all-trans*-retinoic acid and its ester at room temperature are internally converted within the singlet manifold. It should be noted that ϕ_{pc} (pc = photochemistry including photoisomerization) is negligible for these *all-trans*-retinoyl systems as shown by the absence of any permanent change in the region of ground-state absorption following laser excitation.

Discussion

The blue shift of the absorption spectral maxima of retinoic acids on going from 3MP to 3MP + ether, EPA, or 2-methyltetrahydrofuran (2MeTHF) can be best explained in terms of the existence of the acids as dimers in 3MP and as monomers (hydrogen bonded to ether/alcohol molecules) in the other solvents. The IR spectra of *all-trans*-retinoic acid and methyl retinoate (described earlier) very clearly indicate that the acid forms a dimer and/or a hydrogen-bonded complex with ether in solution and that the dimers make use of intermolecular hydrogen bonding involving the hydroxyl and carbonyl group. Carboxylic acids such as acetic acid, benzoic acid,[21,23] and naphthoic acid[24] are well known to form a stable dimer involving two intermolecular hydrogen bonds with a high association constant (5×10^3 to 10^4 M^{-1}). From the estimated value (1×10^4 M^{-1}) for the equilibrium constant of dimer formation of *all-trans*-retinoic acid, about 50% of the acid molecules are associated to form dimers in 3MP at room temperature with 10^{-4} M total acid concentration. Although we have not obtained the enthalpy change, ΔH, for the dimer formation, it may be estimated to be −10 kcal/mol, from analogy with the enthalpy data available for benzoic and naphthoic acid. At 77 K, then, the equilibrium constant is expected to be very high and the molecules are expected to be completely associated as dimers. This is reflected in the observation that Beer's law is obeyed in the concentration range 2×10^{-4} to 5×10^{-5} M at 77 K. At 380 nm, the extinction coefficient, calculated on the basis of monomer concentration, is 5.4×10^4 M^{-1} cm^{-1} for *all-trans*-retinoic acid in 3MP at 77 K.

In analogy to the carboxylic acids, the structures of the dimers of retinoic acids that are predominantly formed in 3MP (or other hydrocarbon solvents) are as follows.

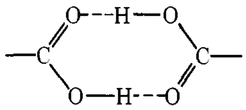


In addition to the well-defined tail-to-tail dimers (shown above), there are other possible forms of aggregates, e.g., dimers involving one hydrogen bond (not necessarily linear), stacked or partially stacked dimers, and *n*-mers of higher order (i.e., $n > 2$). While we cannot rule out the possibility of the existence of these various kinds of aggregates, it appears that, even if present, they do not contribute significantly to the spectral behavior of the systems under the conditions of our experiments. This conclusion is based on the following detailed observations made in the cases of *all-trans*-retinoic acid and *all-trans*-methyl retinoate in 3MP at 77 K in the concentration range 10^{-4}–10^{-5} M. (1) The absorption spectra and molecular extinction coefficients are independent of concentrations. (2) The shape and position of the emission spectra are independent of concentration and excitation wavelengths. (3) The shape and position of the excitation spectra are independent of concentration and monitoring wavelengths. (4) Finally, the emission quantum yields and lifetimes are constant within the experimental errors over the concentration range studied and are essentially independent of excitation wavelengths. All these observations suggest that, even if more than one kind of aggregate is present as a major species, their formation constants, spectral properties, and photophysical properties are essentially identical.

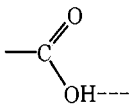
Preliminary results on lifetimes and quantum yields of *all-trans*-retinoic acid indicated that an exciton resonance model gave a very good picture of the electronic structure of the dimer.[4] For it was found that application of this model to the linear tail-to-tail dimers of retinoic acid predicted $\tau_{0,\text{monomer}}/\tau_{0,\text{dimer}} = 2$, which agreed with the initial results. However, during the present work it was discovered that these preliminary experimental values[4] of lifetimes and quantum yields were unreliable and that the new values (see data marked with superscript "e" in Table I) were inconsistent with the simple version of the exciton theory. In order to arrive at a better understanding of the dimer electronic states, a series of experiments were done on retinoic acid with a variety of hydrogen-bonding agents in an attempt to isolate the role of hydrogen bonding in the retinoic acid dimer itself.

From the data in Table I on *all-trans*-retinoic acid with various hydrogen-bonding agents, it can be seen that the results fall fairly cleanly into two classes of hydrogen-bonded complexes on the basis of their photophysical properties. The first class, consisting of retinoic acid dimers (pure 3MP solvent), retinoic acid–acetic acid complexes, and retinoic acid–trichloroacetic acid complexes, have radiative rate constants (k_r) approximately equal to or greater than 2×10^8 s^{-1} and nonradiative rate constants (k_{nr}) greater than 3×10^8 s^{-1}. The second class, which includes the remaining four entries in Table I under *all-trans*-retinoic acid, is characterized by complexes having both k_r and k_{nr} less than 10^8 s^{-1}. In fact, of the eight rate constants coming from the second class of complexes, seven of them fall in the narrow range between 0.58×10^8 and 0.77×10^8 s^{-1}. Only $k_r = 0.26 \times 10^8$ s^{-1} for the triethylamine complex with retinoic acid falls outside of this range.

This breakdown into classes of complexes based on photophysical properties correlates very well with an analogous classification based on differences in the structure of the various hydrogen-bonded complexes involved. In the first class of complexes, all are likely to form two hydrogen bonds in a tail-to-tail fashion.



More importantly, one of these hydrogen bonds directly involves the oxygen that is in conjugation with the rest of the polyene chain. In the second class of hydrogen-bonded complexes, it is only the -OH part of the retinoic acid that is involved in the hydrogen bond. This hydroxyl oxygen is not involved in the π conjugation in the dominant resonance structure of retinoic acid (see later discussion).



X = O,N

The same type of correlation between the structure of the complexes and their photophysical properties holds reasonably well when applied to molecules similar to *all-trans*-retinoic acid. Although the actual magnitudes involved are somewhat different than for the case of the all-trans isomer, both 9-*cis*-retinoic acid and 13-*cis*-retinoic acid dimers have larger k_r's and k_{nr}'s than their corresponding ether complexes. The structural differences between the retinoic acids and *all-trans*-methyl retinoate make this ester an interesting check on the correlation under consideration. Since the ester lacks an -OH group, it is not expected to form a hydrogen-bonded dimer or to form a hydrogen-bonded complex with ether. From Table I, it can be seen that the values for k_r and k_{nr} for the ester in 3MP and 3MP + 10% ether are much closer to the rate constants for the second class of complexes of retinoic acid than to the rate constants for the first class of retinoic acid complexes. On the other hand, the proton donors, acetic acid and trichloroacetic acid, can form hydrogen-bonded complexes with the ester where there is only one single hydrogen bond. Since the carbonyl oxygen is involved in the hydrogen bond, it might be expected that these complexes would have properties similar to those of the first class of *all-trans*-retinoic acid complexes. This can be seen to be true for the trichloroacetic acid complex from Table I. Only the acetic acid-methyl retinoate complex falls outside the correlation. The rate constants for this complex closely resemble the rate constants of the uncomplexed ester. So, if the correlation between the structure of these complexes and the photophysical rate constants are to hold for all cases, acetic acid does not form a very strong hydrogen bond with methyl retinoate.

This correlation is of significance for the electronic structure of the dimers and the hydrogen-bonded complexes since the photophysical rate constants reflect the nature of the fluorescing state. The quantitative agreement between the photophysical rate constants of retinoic acid dimers and of retinoic acid complexes with other carboxylic acids strongly suggests that the hydrogen bonding and not the exciton resonance is of prime importance in the excited states of the retinoic acid dimers themselves. In fact, since hydrogen bonding has such a large effect on the chromophore in retinoic acid, it would be necessary to start with hydrogen-bonded monomers in order to have appropriate zero-order states for the exciton model. In the traditional exciton model, the two monomers are usually taken to interact by the induced dipole–induced dipole interaction. However, in dimers with hydrogen bonds as part of the chromophores, the weakest long-range interaction that could come close to describing the interacting monomers would be dipole–dipole. This case of dimers with hydrogen bonds directly on the chromophore is further complicated by charge transfer between monomers. To carry out such a generalized exciton calculation would require the choice of a reasonable hydrogen-bonded monomer. There is no obvious a priori choice for this hypothetical monomer. Thus, although some type of delocalized wave function is required by the symmetry of the dimer, the most urgent problem of retinoic acid is to understand the zero-order states in some detail.

One technique that has proved useful for this purpose in the polyenes has been two-photon absorption. However, in spite of the fact that the retinoyl systems have high fluoroescence quantum yields, no two-photon excitation spectral study has so far been reported. However, two-photon excitation spectra and other evidence show the existence of a relatively forbidden $^1A_g{}^*$ state as the lowest excited singlet state or one of the low-lying excited singlet states in retinol,[10,14] retinal,[15] and related polyene systems.[2a] A survey of the lifetime data (Table I) shows that the intrinsic radiative lifetimes of methyl *all-trans*-retinoate and retinoic acids (hydrogen bonded to ether, alcohol, or triethylamine) deviate from the lifetimes based on the integrated area of the main absorption band (~1 ns) by one order of magnitude or more. This strongly suggests that the state responsible for fluorescence in the retinoyl systems is also of relatively forbidden nature. By analogy to other polyene systems we may assign this state to be of "$^1A_g{}^*$" character. The state order with $^1A_g{}^*$ state as the lowest state is also consistent with the fact that in these systems the variations in the magnitude of the hydrogen-bond strength can strongly shift the $^1B_u \leftarrow {}^1A_g$ transition in absorption, but do little to shift the emission. The fact that the deviation of the intrinsic radiative lifetimes from those expected for the $^1B_u \leftarrow {}^1A_g$ transition (responsible for the main absorption band) is not as large in the case of retinoic acids as it is in retinols and long-chain polyenals (e.g., C_{22} and C_{24} aldehydes)[18] indicates that the $^1A_g{}^*$ state is relatively close to the ^1B_u state in retinoic acids and hence can borrow intensity from the ^1B_u state. However, it is also possible that the COOR group (R = CH_3, H) breaks the symmetry of the retinoyl systems to a greater extent than the CHO group in polyenals, and this might be responsible for higher intrinsic intensity of the $^1A_g{}^* \leftarrow {}^1A_g$ transition in the former.

With this assumption for the state order in a hypothetical retinoic acid monomer, it is possible to understand some of the photophysics of the dimers and hydrogen-bonded complexes. Partial protonation at the =O site in the conjugated chain would likely red shift the $^1B_u \leftarrow {}^1A_g$ transition. This is actually seen in absorption (if methyl retinoate is taken as equivalent to the non-hydrogen-bonded monomer) and is supported by calculations on the protonated Schiff base.[26] This could increase the vibronic coupling between the excited $^1A_g{}^*$ and ^1B_u states and thus increase the radiative intensity of a complex or dimer having the =O involved in a hydrogen bond. In the complexes where only the -OH is involved in a hydrogen bond, the effect on the ^1B_u state should be much less since this oxygen is not directly part of the conjugation. In general there will be only minor inductive effects. However, in the case of triethylamine, where there is a new resonance structure with π electrons delocalized over both oxygens, there is a decrease in the radiative rate constant relative to all of the other complexes. However, if the methyl retinoate in 3MP is taken as the hypothetical retinoic acid monomer, it is seen that the complexes that form hydrogen bonds with the -OH part of *all-trans*-retinoic acid all have a decrease in their radiative rate constants. With the ester as the monomer standard, the triethylamine complex is only an extreme case of the class II type complexes. For this picture of the behavior of the radiative rate constants to be quantitative, configuration interaction of these mixed vibronic states with charge-transfer states would have to be added along with symmetry considerations for the dimers.

It is interesting to note that dimer formation and hydrogen bonding involving carbonyl oxygen (with acetic/trichloroacetic acid) result in an increase not only in the radiative rate constants (k_r) but also in the nonradiative rate constants (k_{nr}), Table I. As a matter of fact, the effect on k_{nr} is more pronounced, and this results in an overall moderate decrease in ϕ_F. The intersystem crossing,[27] measured in terms of ϕ_{T_1}, is found to be practically zero in the retinoyl systems at room temperature. Also, ϕ_{T_1} remains negligible for these systems upon cooling to −100 °C.[30] This is indicative of the relatively insignificant contribution of k_{isc} to k_{nr} in the temperature range 298–173 K, and is probably a reflection of the fact that $^{1,3}(n,\pi^*)$ states, shown to be involved in the efficient intersystem crossing processes of retinals and their homologues,[20] are located at energies too high for them to be involved in the intersystem crossing in the retinoyl systems. We have not made any attempt to determine ϕ_{T_1} at 77 K. However, we would not expect ϕ_{T_1} of retinoyl systems to be significantly large at 77 K, because the state order in regard to the $^{1,3}(n,\pi^*)$ states would be even more unfavorable for participation in intersystem crossing at the low temperature (77 K). Thus, the observed increase in k_{nr} of retinoyl systems upon formation of a dimer or hydrogen-bonded species (including carbonyl oxygen) at 77 K is primarily associated with an increase in k_{ic} (ic = internal conversion).

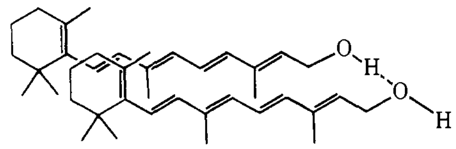
In conclusion, it can be seen that the hydrogen bonding directly to the atoms in the conjugated chains has a profound

Table II. Spectral and Lifetime Data for Monomer and Dimers of *all-trans*-Retinal, -Retinol, and -Retinoic Acid at 77 K

compd	species	solvent	absorption max nm	emission max nm	quantum yield	obsd lifetime, ns	natural radiative lifetime, ns	natural nonradiative lifetime, ns
all-trans-retinal[a]	monomer	3MP	385		$<10^{-4}$			
	monomer, hydrogen bonded with phenol	3MP + phenol	420	600	0.07	1.6	23	1.7
	dimer[c]	3MP	~395	~520	0.05	1.0	20	1.1
all-trans-retinol[b]	monomer, hydrogen bonded with ether	3MP + ether	332.5	482	0.45	15.0	33	27
	dimer[d]	3MP	322	484	0.25	15.1	60	20
all-trans-retinoic acid	monomer, hydrogen bonded with ether	3MP + ether	364	468	0.54	8.0	14.8	17
	dimer	3MP	381	470	0.44	1.7	3.9	3.0

[a] From ref 3 and 4. [b] From ref 10. [c] Proposed structure: partial sandwich (ref 4) or tail-to-tail linear (ref 31). [d] Proposed structure: partially stacked (ref 10) or tail-to-tail with tails pointing in the same direction ("card-stacked").

effect on the photophysics, whereas hydrogen bonding to atoms not in direct conjugation has little effect. In addition, for dimers with intermolecular hydrogen bonds, these effects can be so large that exciton resonance effects are negligible. These two findings have implications for two problems in related molecules. First, in retinal dimers, large mixing with a charge-transfer state was required to bring the fluorescence lifetimes (see Table II) of dimers and monomers into quantitative agreement with exciton theory.[4] It can now be seen that such large mixing of CT states is not necessary because the only "monomers" of retinal that fluoresce are retinal complexes in which the oxygen atom is directly hydrogen bonded to some other agent.[3] Since the photophysical effects are so large for this type of hydrogen bonding, the monomer lifetime should not be used to predict the fluorescence lifetime of the exciton state of the retinal dimer, which is not hydrogen bonded. The second problem involves retinol dimers which have been observed to have (1) blue-shifted absorption spectra,[10] (2) long radiative lifetimes[10] (see Table II), and (3) intermolecular hydrogen bonds.[32] In addition, (4) the finding that hydrogen bonds not involving the chromophores have small photophysical effects suggests that retinol dimers are expected to follow the exciton model. In fact, the exciton model for the "card-stacked" structure



meets all four requirements.

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