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The Isomer Dependence of Oscillator Strengths in Retinal and Related Molecules. Spectroscopic Assignments

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Abstract: The spectroscopic properties of the higher excited states of retinal isomers and related molecules are considered in detail. A simple graphical description is used to characterize the transition moments to various excited states and it is shown that the isomer dependence of oscillator strengths is a particularly reliable parameter for making spectroscopic assignments. We show that the 280-nm (β) band of retinal isomers is the cis band corresponding to a transition to the lowest A_g^+ state. It derives intensity from the s-cis ring chain conformation in all retinal isomers and is further enhanced by cis conformations along the polyene chain. It is particularly intense for conformations with central cis double bonds in keeping with the classical behavior expected for cis bands. Our assignment of the cis band allows us to explain a large number of experimental observations including the appearance of vibrational structure for this band alone at low temperature. The 250-nm (γ) band in retinals is not assigned; however, we show that previous assignments for this transition are incorrect. Based on our results for retinals we are able to identify the cis (β) and γ bands in the spectrum of both rhodopsin and bacteriorhodopsin.

Introduction

Polyene spectroscopy and photochemistry have become areas of considerable interest over the past few years. Much of the impetus has come from interest in retinal isomers which play a central role as chromophores in visual pigments and bacteriorhodopsin.[2] Our major concern in this study is the assignment of the spectroscopic transitions of the higher excited states of retinal isomers. In addition to the intrinsic interest in this controversial problem a proper state assignment is required for the evaluation of theoretical studies of the excited-state manifold of these complex molecules.

It has become common practice in polyene spectroscopy to use symmetry labels appropriate to all-trans isomers which belong to the C_{2h} point group. The main strongly allowed absorption band in all polyenes results from an excitation into the lowest B_u^+ state. At approximately the same energy, although generally at longer wavelengths, a weakly allowed A_g^- state has been identified in a number of polyenes.[3] A proper theoretical description of this state requires the inclusion of multiply excited configurations in an SCF-CI molecular orbital scheme. Another important state which can be easily assigned in many polyenes is the A_g^+ state which corresponds to the "cis" band identified by Zechmeister.[4] In addition to these well-studied transitions, further B_u^-, A_g^-, and B_u^+ states are calculated to be present 1 or more eV above the main absorption band and thus should be considered in any assignment of higher excited states.[5]

In this paper we first review the spectroscopic properties of polyenes and consider the extent to which it is possible to reproduce the relative intensities and transition energies of their observed absorption bands with standard π electron calculations. The isomer dependence of oscillator strengths is then explained qualitatively in terms of localized dipoles positioned at each nucleus and these are used in a qualitative interpretation of polyene spectra. Turning to the absorption spectra of retinals and closely related molecules, we find that the 280-nm band system can best be assigned as the A_g^+ cis band while the third absorption band, which has no obvious analogue in polyene spectra, remains somewhat of a mystery band.

Features of Polyene Spectra

Long polyenes exhibit consistent behavior with respect to both the intensity and position of their three lowest absorption bands.[4] The first optically allowed transition corresponds to the intense B_u^+ state and exhibits a characteristic red shift with increasing chain length.[6] The transition is most intense in trans isomers and is weakened somewhat by the presence of cis bonds in the chain.[4] The second allowed absorption band in long polyenes is the cis band which corresponds to a transition to the A_g^+ state. The transition gains intensity in cis isomers.

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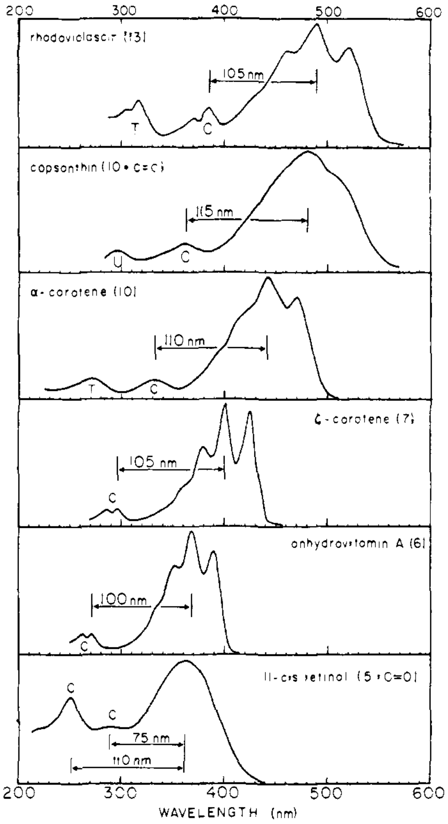

Figure 1. The wavelength separation between the "cis band" and the λ_{max} band as a function of the number of conjugated double bonds in the polyene chain (given in parentheses). Those absorption bands which increase in intensity upon trans → cis isomerization are labeled with a "C". Those bands which, in addition to the λ_{max} band, decrease in intensity upon trans → cis isomerization are labeled with a "T". The label "U" indicates a band which is essentially unchanged by trans → cis isomerization. Rhodoviolascin (*Arch. Biochem.*, **5**, 243 (1944)), α-carotene (*J. Am. Chem. Soc.*, **65**, 1522 (1943)), ζ-carotene (*J. Am. Chem. Soc.*, **76**, 2923 (1954)), and anhydrovitamin A and 11-*cis*-retinal (this work) were taken in hexane solution. Capsanthin (*J. Am. Chem. Soc.*, **66**, 186 (1944)) was taken in benzene solution. All compounds, with the exception of 11-*cis*-retinal, are mixtures of cis and trans isomers.

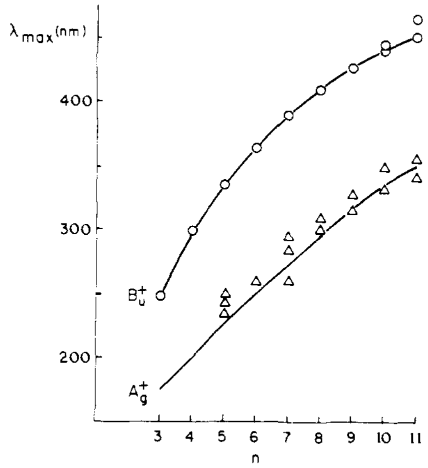

Figure 2. Calculated and experimental values of λ_{max} for a series of polyenes with n = 3-11 double bonds. Solid lines are theoretical curves while open circles and triangles are experimental points for the B_u^+ and A_g^+ states, respectively. PPP-SCF-MO calculations included all singly excited configurations. The resonance integral is calculated from $\beta = \frac{1}{2}(\beta_i^\circ + \beta_j^\circ)S_{ij}$, where β° = 10.15 eV. The ionization potential for carbon is taken as 10.9 eV and the one-center Coulomb integral is 11.11 eV. The Mataga approximation was used to calculate the two-center Coulomb integrals. For the B_u^+ state λ_{max} corresponds to the first member of the stretching progression for $N < 8$ with the exception of $n = 3$. For $n = 3$ and $n > 8$, the second peak was used; n = 3, 4, 6, 8, 10 from ref 6; n = 5, 7 from ref 9; n = 9, 11 from ref 4. For the A_g^+ state all values were taken from J. Dale, *Acta Chem. Scand.*, **8**, 1235 (1954).

particularly s-cis, and becomes more intense as the cis bond approaches the central position along the chain. The latter observation appears to be an excellent rule of thumb for long polyenes.[4] The cis band also is red shifted with increasing chain length as can be seen from Figure 1, where it is the central "C" band.

The third symmetry-allowed absorption band characterized by Zechmeister is separated by about 13 000-15 000 cm^{-1} from the main transition. It corresponds to a higher B_u^+ state and exhibits an isomer intensity dependence similar to that of the main B_u^+ transition. It is most intense for trans isomers and loses intensity as cis linkages are introduced into the chain. (See the short-wavelength T band in Figure 1.)

The data for polyenes with four to seven double bonds are less reliable since the dependence of band intensity on isomer composition has not been systematically studied. The best data[4,7] exist for "retro" compounds where the cis band is separated by about 10 000-11 000 cm^{-1} from the main band, and for diphenylpolyenes[4] where the separation is also about 10 000 cm^{-1}. The naturally occurring pentaene, phytofluene,[4] also has a separation of 10 000 cm^{-1} as does axerophtene,[8] a hydrocarbon analogue of retinal. The hexaene anhydrovitamin A[8] has a 10 000-cm^{-1} separation while a synthetic heptaene[9] has been synthesized with a separation of about 9000 cm^{-1}. In general the energy separation between the main band and the cis band increases in shorter polyenes leading to the nearly constant separation in wavelength of about 110 nm (see, for example, Figure 1). It should be emphasized that the determination of the various band separations is rather unreliable when vibrational fine structure cannot be identified. Clearly band origins provide the best criterion, but these are generally unavailable.

Theoretical Studies of Polyene Spectra

Energy Levels. An extensive theoretical discussion of the excited states of polyenes has recently been published by Schulten, Ohmine, and Karplus.[5] These workers have emphasized the presence of optically forbidden "covalent" states in the range of the observed "ionic" B_u^+ and A_g^+ states. They show that multiply excited configurations must be included in the framework of SCF π electron theory to account for the relative positions of the covalent states. Unfortunately, quantitative agreement appears difficult to obtain, even for small polyenes such as hexatriene.[10] On the other hand, the effect of multiple excitations on the optically allowed B_u^+ and A_g^+ states is not severe,[5] thus accounting for the ability of standard PPP theory to provide a satisfactory explanation of the band systems observed in polyene spectra.

Our major interest in this work is to assign the observed spectroscopic transitions of retinals and related molecules. It seems reasonable therefore to first determine the extent to which the optically allowed "ionic" states provide plausible assignments and this may be done without resorting to multiple excitations.

Calculations[11] of λ_{max} vs. chain length are summarized in Figure 2 for the two lowest optically allowed transitions in polyenes. There is excellent agreement with experiment for the B_u^+ state for a large sample of molecules. Agreement for the A_g^+ state is also good, although the calculated values tend to slightly overestimate the transition energy. It is expected that the results for the main band be the best since the parameters were fit for this transition.

Intensities. Calculations of oscillator strengths are generally highly inaccurate in absolute value. However, in the following we will be concerned primarily with the sensitivity of the var-

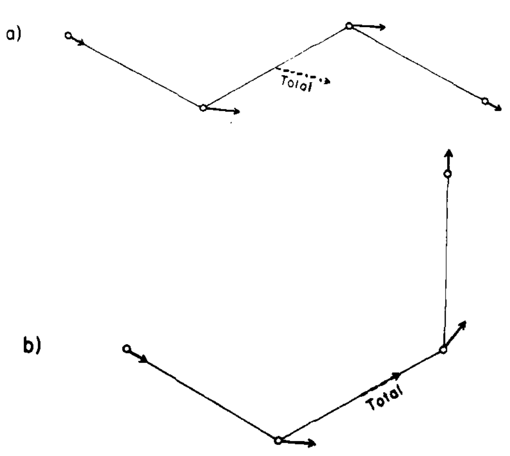

Figure 3. Local atomic transition dipoles for *trans*- and *cis*-butadiene calculated from eq 2 for the main $\mathrm{B_u}^+$ transition. Note that a rigid rotation of the local moments of the trans isomer of Figure 3a about the 2-3 bond leads almost exactly to those calculated in Figure 3b. The reduced transition dipole in *cis*-butadiene is purely a function of geometry.

ious absorption bands to isomer composition so that relative rather than absolute oscillator strengths are of interest. It is of importance to determine whether semiempirical methods may be expected to provide a reliable description of the conformational dependence of the transition dipoles of polyenes, to understand why the "rules of thumb" provided by Zechmeister (see above) work so well for carotenes, and to decide whether they may be applied with confidence to other polyenes.

Early rationalizations of the isomer dependence of carotene spectra were based on semiclassical theories in which each transition corresponded to a mode of oscillation of an electronic charge along the polyene chain.[4] The different modes were described graphically and this allowed a clear distinction among the different transitions. This extremely useful type of classification may now be based on more rigorous quantum-mechanical considerations.

Recently[12] it has been pointed out that transition movements may be rigorously written in the form

$$P_{nm} = \sum d^i{}_{nm} = \omega_{nm}\langle m|\sum r_i|n\rangle = -(h/m_e)\langle m\,|\sum\nabla_{R_i}|n\rangle \qquad (1)$$

where ω_{nm} is the transition frequency, n_e is the number of electrons, n_n is the number of nuclei, and ∇_{R_i} is the gradient operator with respect to the coordinates of the ith nucleus. It is apparent from eq 1 that each electronic transition is composed of localized nuclear contributions. The $\mathbf{d}^i$'s involve differentiation of the transition density and thus their magnitude depends on the sensitivity of the transition density to the displacement of the ith nucleus.

Within the context of the ZDO approximation, the contribution to $d^i{}_{nm}$ from a single electron excitation from orbital l to orbital k is given by

$$d^i{}_{l\to k} = (\sqrt{2}/2)\sum (C_{lj}C_{kj} - C_{li}C_{kj})\langle i|\nabla|j\rangle \qquad (2)$$

In eq 2 ∇ operates on the electronic rather than nuclear coordinates. The C_{lj} are the coefficients of the jth atomic orbital k, in the lth molecular orbital. The integrals $\langle i|\nabla|j\rangle$ were calculated as in ref 13. When carrying out the CI calculations, the $d^i{}_{nm}$'s for each configuration have to be summed to yield the total d^i for the transition. A typical situation is shown for the first transition in butadiene (Figure 3). The arrows on each nucleus are the localized dipoles which are calculated to be larger for the central atoms than for the terminal ones. Note that the cis transition moment can be almost exactly obtained from the trans by a rigid rotation of the localized dipoles. Dipoles for five transitions in *trans*-dodecahexaene have been summed in Figure 4 over half or smaller fractions of the molecule so as to provide a schematic representation of each transition which will be used below.

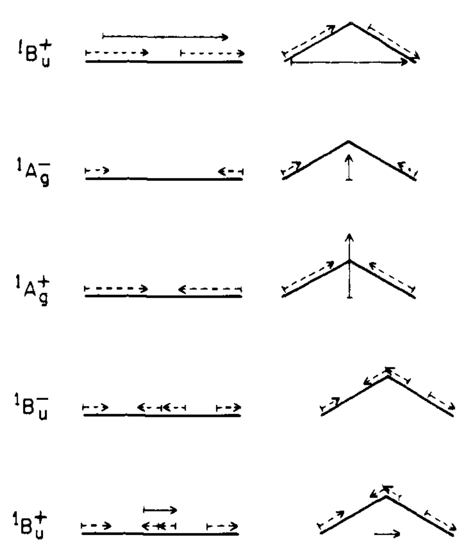

Figure 4. Schematic diagram of resultant transition dipoles to various excited states in dodecahexaene. The heavy solid line designates the molecular axis while the dashed arrows refer to local dipoles summed over half or smaller fractions of the molecule. The arrow drawn with solid lines, where present, denotes the net transition moment. The first column refers to the all-trans isomer while the second refers to a cis isomer about a central double bond.

Isomer Dependence of Transition Moments. It is generally the case for polyenes that molecular orbitals are relatively insensitive to changes in geometric configuration, particularly for planar isomers. As a result, if one rigidly transforms the all-trans isomer to a cis isomer by a bond rotation, then the localized dipoles are unchanged but are rotated by the same transformation. This is illustrated for *cis*- and *trans*-butadiene in Figure 3.

Assuming the existence of fixed local dipoles, conformational effects on different electronic states may be easily deduced. Thus, the $\mathrm{B_u}^+$ transition is expected to lose intensity in going from a trans to cis conformation simply because the net moment in the direction of the long axis of the molecule is reduced. The effect is of course largest for central cis linkages (see Figure 4). The $\mathrm{A_g}^+$ state exhibits essentially reversed behavior, having zero oscillator strength in trans isomers and gaining maximum intensity in isomers with central cis linkages. Thus, it corresponds to the cis band characterized by Zechmeister.[4] The $\mathrm{A_g}^-$ state also exhibits some of the characteristic behavior of a cis band. If differs from the $\mathrm{A_g}^+$ state in the magnitude of the various local dipoles, but not in the geometry dependence of the entire molecule.

In another example, the $\mathrm{B_u}^-$ state is forbidden in polyenes both for trans isomers and central cis isomers. This follows since each half of the molecule has a zero net dipole (Figure 4). However, the $\mathrm{B_u}^-$ state should become allowed for noncentral cis linkages and thus is not a cis band in the classical sense. (It is interesting to note that the $\mathrm{B_u}^-$ state behaves like a cis band under two photon selection rules.[22b]) Finally, the second $\mathrm{B_u}^+$ state behaves qualitatively like the main $\mathrm{B_u}^+$ transition and is most intense in trans isomers. Thus, it corresponds to the third absorption band characterized by Zechmeister in carotene spectra (see Figure 1).

Most of the features that characterize the various electronic states in polyenes are still present in the polyene aldehydes even though the symmetry designations are no longer valid (indeed their absorption spectra[14] are similar to those of polyenes of the same length). The major difference is that the molecule is

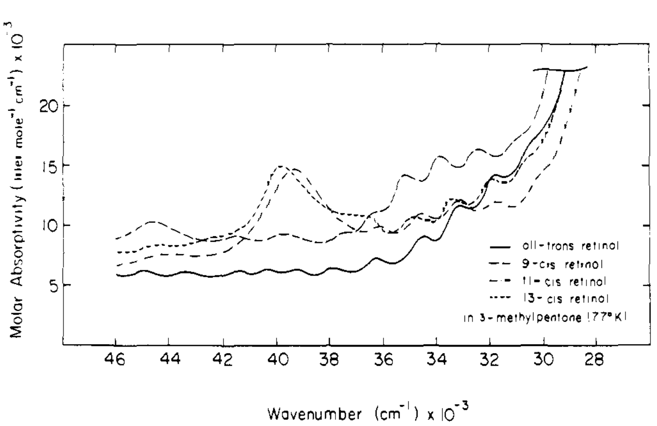

Figure 5. The absorption spectra of *all-trans-*, 9-*cis-*, 11-*cis-*, and 13-*cis-*retinal between 320 and 360 nm.

no longer divided into two equivalent halves so that all transitions become weakly allowed, even in trans isomers (see Figure 4).

We have emphasized the importance of intensity arguments (which have been virtually ignored in recent discussions of retinal spectra) since they provide a particularly strong basis for making spectroscopic assignments. This is the case since *relative* intensities are strong functions of geometry while calculated values of electronic energy are subject to severe uncertainties, and depend strongly on the set of parameters used. This is particularly true for higher excited states as can be seen from Figure 2 and is evident from the fact that parameters are generally picked so as to fit the lowest transition.

Spectra of Retinal Isomers and Related Molecules

General Features. The spectra of retinal like molecules have been extensively studied both at room and low temperatures.[4,15] Retinal itself has a main band at about 370 nm and two subsidiary bands centered at 280 and 250 nm (Figure 1). The 280-nm band exhibits vibrational structure but none is observed in the other two bands. The intensity ordering at room temperature for the main (α) band is trans > 13-cis > 9-cis > 11-cis; 9 > 11 > 13 > trans for the 280-nm (β) band and 11 > 13 > 9 > trans for the 250-nm γ band. At 77 K these relationships are changed somewhat particularly for 11-*cis*-retinal (see Figure 5). However, this isomer exists in two conformeric forms,[16] 12-s-cis and 12-s-trans, at room temperature and their relative proportions are known to change as the temperature is lowered[16b] and in different solvents.[17] Moreover, both conformers are highly twisted, thus complicating the application of the simple rules which are valid for the planar isomers. It appears reasonable then to exclude the 11-cis isomer as a basis for making spectroscopic assignments.

The absorption spectra of retinal Schiff bases have been reported[18] and are quite similar to the aldehyde spectra. Spectra of 11-cis and all-trans protonated Schiff bases are also available[19] and for each a distinct three-band pattern, reminiscent of retinals, is obtained. Unpublished data (R. Callender, private communication) of the 9- and 13-cis protonated Schiff bases also reveal the familiar three-peak pattern with the 9-cis isomer most intense in the β band and the 13-cis isomer most intense in the γ band.

The spectra of 3,4-dehydroretinals[20] (vitamin A2 aldehydes) are quite similar to those of retinals. The intensity orderings of the three bands are essentially identical, with the 9-cis isomer becoming particularly intense for the β band. However, all isomers gain intensity in this region of the spectrum.

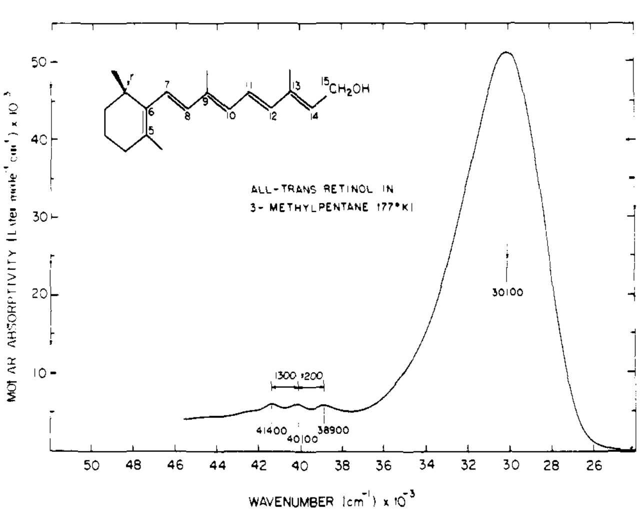

Figure 6. The absorption spectrum of *all-trans*-retinol in 3-methylpentane at 77 K.

The final series of molecules that needs to be considered are the retinols.[20] In retinol isomers the terminal heteroatom is no longer conjugated with the polyene chain so that to a first approximation they may be regarded as pure polyene hydrocarbons. *all-trans*-Retinol has a weak β band that is hard to resolve at room temperature but is evident at 77 K (Figure 6). The 9-cis isomer has the most intense β band while 11-*cis*-retinol, which absorbs weakly in this region, appears to have an intense γ-band (although it is not clear whether this is not a β band shifted for some reason to slightly longer wavelengths). Unfortunately, no low-temperature spectra for this isomer are available.

The spectra of a number of A2 alcohols are also available[20] and all have an intense β band and no obvious γ band. The intensity ordering for the β band is 9 > 13 ~ all-trans (no 11-cis spectra are reported).

Spectroscopic Assignments. The β Band. The basic difficulty in assigning the spectral transitions in retinals and related molecules is the presence of two subsidiary absorption bands, both of which gain intensity in going from trans to cis isomers. Most polyenes exhibit only one band in addition to the main band and this is clearly assigned to the A_g^+ state. The longer polyenes (see Figure 1) do have two subsidiary bands but the short-wavelength peak is a B_u^+ state which is most intense in trans, not cis, isomers.

The experimental data show that the 280-nm band gains intensity primarily from the A_g^+ state and thus can best be assigned[4] to the classic "cis peak". The basis for the assignment is the intensity ordering among isomers as well as changes in intensity in going from the Al to A2 series of molecules. Recalling that the 9-cis isomer contains a central cis double bond while the 13-cis isomer contains a terminal double bond it is clear that the β band gains intensity for central double bonds in each series of molecules under discussion. This is the classic behavior expected of the cis band and is in clear contrast to the behavior of the 250-nm γ band, which gains intensity in terminal (13-cis) cis isomers.

In the A2 series a general increase in intensity is expected in the cis band region for all isomers due to the s-cis single bond in the ring. This effect is evident in the β band of A2 alcohols[20] and aldehydes,[20] providing strong additional evidence for assigning the β band to the A_g^+ state. In contrast, the γ band tends to lose intensity in the A2 series.

These qualitative arguments are further supported by the calculated oscillator strengths tabulated in Table I. The 9-cis isomer is found to have the most intense transition to the A_g^+ state in all four classes of molecules as is found experimentally for the β band. In contrast, the 13-cis isomer is predicted to have a relatively weak transition to this state, again in complete

Table I. Calculated Oscillator Strengths[a]

	B_u^+	A_g^-	A_g^+	B_u^-
A_1 retinol				
all-trans	1.7	0.02	0.15	0.01
9-cis	1.3	0.06	0.48	0.01
11-cis (12-s-cis)	1.08	0.09	0.25	0.05
11-cis (12-s-trans)	1.40	0.04	0.20	0.02
A_1 retinal				
all-trans	2	0.08	0.09	0.1
9-cis	1.6	0.15	0.32	0.18
11-cis (12-s-cis)	0.64	0.5	0.6	0.14
11-cis (12-s-trans)	1.3	0.34	0.25	0.06
13-cis	1.8	0.18	0.14	0.06
A_2 retinol				
all-trans	1.7	0.08	0.2	0.01
9-cis	1.3	0.13	0.6	0.01
11-cis (12-s-cis)	1.2	0.14	0.19	0.00
11-cis (12-s-trans)	1.4	0.12	0.25	0.03
A_2 retinal				
all-trans	2.1	0.06	0.15	0.1
9-cis	1.5	0.3	0.47	0.11
11-cis (12-s-cis)	0.74	0.66	0.23	0.14
11-cis (12-s-trans)	1.42	0.32	0.13	0.25
13-cis	1.9	0.16	0.15	0.11

[a] Calculations as in Figure 2. Oxygen parameters are $\beta^\circ = 18.5$ eV, $H_{ii}^\circ = 15.1$ eV, and $\gamma_{ii}^\circ = 13.0$ eV. The ionization potentials and one-center Coulomb integrals were varied according to $h_{ii} = h_{ii}^\circ(Z_{\text{eff}}/Z_{\text{eff}}^\circ)^2$ and $\gamma_{ii} = \gamma_{ii}^\circ(Z_{\text{eff}}/Z_{\text{eff}}^\circ)$ as suggested by M. J. S. Dewar and C. de Llano, *J. Am. Chem. Soc.*, **91**, 789 (1969). The effective nuclear charge Z_{eff} was calculated at each iteration from Brown and Heffernan, *Trans. Faraday Soc.*, **54**, 757 (1958). The ring chain angle was fixed at the representative value of 135° (twisted s-cis) for all molecules. In 11-*cis*-retinal the torsional angle about the 12-13 single bond was taken as 140° for s-cis conformers and 40° for s-trans.

agreement with the observed behavior of the β band. Finally, all isomers are calculated to have a significant increase in intensity for their A_g^+ state in going from the A1 to the A2 series and this striking behavior is evident in the β-band region. No other band system meets these qualitative and quantitative expectations; indeed, the γ band which is the other candidate for the cis band[21] exhibits completely inverse behavior, becoming intense for the 13-cis rather than the 9-cis isomer and losing intensity in going from the A1 to A2 aldehydes. In this regard it is important to recall that in the A2 series the 9-cis isomer has the central cis double bond and should have the strongest cis band.

In light of this evidence, it would appear that arguments against assigning the 280-nm retinal as a cis band would need to discredit intensity considerations as a basis for assignments. However, the discussion above relies on both experimental analogies to carotene spectra and on theoretical calculations, both of which lead to the same conclusion. Moreover, we have shown that relative oscillator strengths are a particularly sensitive measure to use in assignments. We conclude then that the 280-nm band in retinals corresponds to the true A_g cis band.

In a recent paper[21] it was concluded that the 250-nm γ band corresponds to the cis band based primarily on the transition energy obtained from PPP calculations which placed the A_g^+ state of 251 nm, i.e., at the observed wavelength of the γ band. The arguments presented in this section preclude this assignment; however, it is important to emphasize the more general point made above that absolute transition energies should not by themselves be used for assignments. From Figure 2, for example, it is evident that parameters that yield perfect agreement with experiment for the main band can be off by about 20 nm for the subsidiary band. Second, the effect of multiple excitations was not taken into account in ref 21 and these can have significant effects on calculated transition energies. Third, substituent effects are not in general included in PPP calculations and here again significant (10-20 nm) shifts are to be expected. Fourth, the calculations reported in ref 21 yield 351 nm for the B_u state and 251 nm for the A_g^+ transition. Since the main band is in error by about 20 nm it seems unlikely that the calculations for the A_g^+ state transition are accurate. In fact, given the error for the main band, the calculated value (251 nm) for the A_g^+ transition would appear to better fit the 280-nm absorption band, as suggested in this work. Indeed more accurate calculations[22] which include doubly excited configurations place the A_g^+ state at about 265 nm, between the β and γ bands. Since the inclusion of triply excited configurations and the addition of substituent effects would both be expected to further lower the transition energy, theoretical calculations, if anything, support our assignment of the 280-nm β band as the transition to the A_g^+ state.

The only difficulty associated with our assignment that we are aware of is that the energy gap between the main band and the 280-nm band in retinals is about 7500 cm^{-1}, close to that seen in carotenes but less than the 10 000 cm^{-1} observed for polyenes of the same length (see above) such as anhydrovitamin A.[8] We have no definitive explanation for this apparent anomaly, but a number of factors may be involved. First we note that the γ band is separated by about 13 000 cm^{-1} from the main band. Thus, the gap for the γ band is about 3000 cm^{-1} greater than that for the cis band in anhydrovitamin A while that of the β band is smaller by about 2500 cm^{-1}. Since there is no γ band in most polyenes, it is possible that a splitting between the A_g^+ state and the state that gives rise to the γ band (see below) is responsible for the observed transition energies. A second possibility is that another band underneath the cis band contributes intensity in the 280-nm region and gives rise to the unusual band gap. This, however, seems unlikely since the 280-nm band is the only one which exhibits vibrational structure and this is suggestive of a pure state. A third factor may result from the fact that retinals have a s-cis ring-chain conformation while in anhydrovitamin A the ring and chain are connected by a double bond. Since s-cis conformers are known to have significant effects on absorption spectra[16a] the cis band in retinals may be red shifted somewhat. A final possibility is that the α and β bands derive intensity from different conformers with different absorption profiles. This will be considered more fully in the next section.

Vibrational Structure. As is evident from Figure 5 and has been noted previously, the β band exhibits a clearly discernible vibrational progression which would appear to correspond to a C—C stretching mode. Other π-π^* transitions in retinal have no fine structure and it is important to consider the factors that might give rise to vibrational structure. The absence of structure in the main band has been extensively studied, both experimentally[8] and theoretically,[2c,23] and is due to the unusually broad torsional potential for twisting about the 6-7 single bond. Since many conformers (primarily s-cis) with different values for their torsional angle about this bond are expected to be present, even at low temperature, absorption bands will in general be broadened (see discussion in ref 24).

The effect on the main band may be understood from Table II. Twisting from 150 to 90° (a range over which the potential is essentially flat[2c,23]) blue shifts the main band by about 2000 cm^{-1} with only a small effect on oscillator strengths. This amount is more than sufficient to obliterate the vibrational structure inherent in the spectrum of a single molecule. The effect of twisting on the A_g^+ cis band transition is significantly different. The blue shift in going from 150 to 90° is of comparable magnitude to that of the B_u^+ state, but, in contrast to the main band, the effect on the oscillator strength is significant. Even for 9-*cis*-retinal, which derives much of its cis in-

Table II. The Effect of Ring-Chain Twisting on Calculated[a] Transition Energies and Oscillator Strengths

	all-trans		9-cis		13-cis	
$\phi_{6\text{-}7}$	B_u^+	A_g^+	B_u^+	A_g^+	B_u^+	A_g^+
0	367 (2.3)	251 (0.01)	368 (2.0)	254 (0.19)	368 (2.1)	253 (0.05)
30	354 (2.2)	244 (0.01)	355 (1.9)	247 (0.14)	355 (1.9)	245 (0.05)
90	335 (1.9)	228 (0.00)	337 (1.8)	229 (0.06)	336 (1.8)	228 (0.01)
150	355 (2.0)	246 (0.05)	356 (1.7)	249 (0.25)	356 (1.8)	248 (0.09)
180	372 (2.0)	225 (0.11)	374 (1.6)	258 (0.43)	372 (1.8)	256 (0.13)

[a] Calculations as in Table I. Oscillator strengths in parentheses.

tensity from the 9-10 cis double bond of the chain, ring-chain twisting has a major effect on the oscillator strengths. The A_g^+ state gains intensity as the ring approaches the planar s-cis (180°) configuration while more twisted conformers have markedly reduced oscillator strengths.

The presence of fine structure in the β band may be understood in these terms. Most of the intensity for the transition comes from the more planar s-cis conformers and this results in a smaller degree of torsionally induced inhomogeneous broadening in this band relative to the other transitions where all conformers contribute approximately equal intensity.

The effect of ring-chain twisting also provides a factor that may contribute to the small energy gap between the main band and the β band in retinal isomers, relative to other polyenes of comparable length. Most of the intensity for the cis band is derived from nearly planar conformers, while all conformers, with a slight preference for highly twisted ones, contribute to the main band. Since the more planar retinal isomers absorb at longer wavelengths, a significant reduction in the apparent energy gap will be observed relative to planar polyenes where inhomogeneous broadening is not a factor. Moreover, as mentioned above, the red shift of the cis band in retinals compared to say, anhydrovitamin A may result in part from the fact that retinals derive intensity from s-cis conformers about the 6-7 single bond while the cis band in anhydrovitamin A is due to a double-bond isomer. Since the β band derives intensity from more planar isomers than the α band, the magnitude of the s-cis red shift will be larger for the β band, and a decrease in the apparent energy gap, relative to anhydrovitamin A, will result.

The 250-nm "γ" Band. The three-band pattern observed in retinals is characteristic of no other polyenes. (As mentioned above, the third band in carotenes is a high-energy B_u^+ state which is most intense in trans isomers and thus cannot be identified with the γ band.) The γ band is also observed in vitamin A2 aldehydes but is absent in the A2 alcohols.[20] It is also present in protonated Schiff bases,[19] suggesting that a heteroatom conjugated with the polyene chain must be present if it is to be observed. (The single exception to this conclusion is 11-*cis*-retinol, which seems to have a γ band even though the terminal oxygen is not conjugated; however, the spectrum is diffuse and given the instability of the alcohols it seems worth carrying out a low-temperature study of this molecule.)

As mentioned above, the γ band is quite intense in 13-cis isomers and weak in 9-cis isomers and this should provide an important clue as to its identity. We have carried out CNDO/S calculations using doubly excited configurations in the CI scheme in the hope of identifying a transition that was particularly strong in 13-cis isomers and weak in 9-cis and all-trans isomers. The A_g^- state partially meets this criterion but it has recently been shown by two-photon spectroscopy[25] to lie under the main band, in agreement with theoretical calculations,[22] and thus cannot be the γ band. A number of higher A_g and B_u states are calculated to lie in the region appropriate to the γ band and to gain intensity in the 13-cis isomer (in particular the possible presence of 13-cis 14-s-cis isomers[26] could account for intensity in the 250-nm region). Unfortunately, uncertainties in the calculated transition energies make it difficult to distinguish between the various possibilities.

Discussion

Other Assignments. A number of other assignments have recently been made for the 280-nm band and it is of use to reconsider these studies in light of our own conclusions. Birge and co-workers suggested, for example, that the solvent shift behavior and vibronic structure of the 280-nm band could best be rationalized by assigning it to the n-π^* transition (see footnote 6 of ref 17 and ref 27). However, this assignment is unlikely to be correct since vitamin A1 and A2 alcohols, as well as protonated Schiff bases, all have characteristic β bands even though they have no nonbonding electrons. Moreover, the observed intensity ordering among isomers is difficult to interpret in terms of an n-π^* transition which is local in nature and should be only marginally affected by the geometry of the chromophore.

Das and Becker[21] have recently published an extensive spectroscopic study of polyenals and polyenones related to retinals but whose polyene chain length was varied. Since we feel that their data support our assignment of the 280-nm band and that their own interpretation is unlikely to be correct, it is important to consider their results in some detail. Das and Becker assign the 250-nm γ band as the cis band based on their calculations of the transition energy to the A_g^+ state. However, as discussed above, standard PPP calculations are not reliable enough to be used alone as a basis for assignments and indeed the body of evidence discussed in our paper precludes the assignment of Das and Becker. The 280-nm band is assigned in their work to absorption due to 6 s-trans conformers present in solution to the extent of ~10% in equilibrium with the more stable distorted 6 s-cis conformers. They list a number of observations to support their assignment, yet each of these can more easily be explained if, as we suggest, the 280-nm band is the cis band. Moreover, additional data they report are in conflict with their assignment but are consistent with our assignment of the 280-nm band as a cis band. The relevant observations and interpretations are discussed in the following paragraphs.

(a) The fine structure in the 280-nm band has been discussed above and is completely consistent with our assignment.

(b) There is a lack of a β band in the series $CH_3(CH{=}CH)_xCHO$. This results from the absence of a β-ionone ring in these molecules since it is the 6 s-cis conformation that accounts for the intensity of the cis band. In the absence of the ring no cis intensity should be seen.

(c) The polarization direction of the β band is different than that of the main band. This follows from the well-known observation that the cis band is polarized in a perpendicular direction to the main band.

(d) There is a decrease in intensity in the β band along a series of polyenals of increasing chain length while the intensity of the α band increases. This can be understood qualitatively from geometric considerations since the size of the perpendicular component due to the 6-s-cis conformation relative to the long axis of the molecule decreases with increasing chain length. Since the A_g^+ state is polarized in this perpendicular direction and gains intensity from the 6-s-cis bond, its intensity should decrease for longer polyenes. These expectations are borne out by theoretical calculations. We have calculated oscillator strengths (as in Figure 2) for the B_u^+ and A_g^+ states for a series of polyenes where the terminal single bond was fixed at a twisted s-cis conformation (45°) so as to simulate the series of molecules whose spectra appear in Figure 2 of ref 21. For the all-trans isomers we find 0.30, 0.63, 1.1, and 1.7 for the B_u^+ transition and 0.74, 0.35, 0.23, and 0.15 for the A_g^+ transition, for $n = 2, 3, 4$, and 5 double bonds, respectively. It is clear that the trend observed by Das and Becker is reproduced in these calculations if the β band results, as we suggest, from a transition to the A_g^+ state.

(e) An additional trend, not explained by Das and Becker, is that for each molecule considered the β band has a higher oscillator strength for a cis isomer than for the corresponding trans isomer. This of course is completely consistent with the β band being a cis band but is difficult to understand in terms of their assignment since there is no obvious reason for 6-s-trans conformers to be preferred for isomers that have cis double bonds further along the chain.

(f) The α band shifts to the red upon lowering the temperature while the β band displays a much smaller temperature dependence. This follows from the discussion of vibrational structure given above (see Table II). The β band derives most of its intensity from conformers with small values for the 6-7 torsional angle while all conformers contribute almost equally to the α band. At low temperature more planar conformers are preferred and these will necessarily red shift the α band but will have little effect on the β band, which in any case derives most of its intensity from the planar forms. The assignment of Das and Becker is inconsistent with the temperature dependence since their assumption of an equilibrium population of 10% s-trans conformers at room temperature implies a population of 0.01% at 77 K. If this were the case the β band should disappear at low temperature, but in fact the oscillator strength of this band is essentially independent of temperature.

(g) It is difficult to understand why the transition to the allowed B_u^+ state in 6-s-trans conformers should appear at 280 nm as required by the model of Das and Becker (when compared to ~370 nm for the s-cis conformers). In fact, the maximum blue shift expected for a completely twisted ring would be to about 330 nm, corresponding to the absorption of a pentaene (see Figure 2).

Pigments. Finally, it is of interest to consider the location of the higher excited states of visual pigments and bacteriorhodopsin (bR) in light of our results. As mentioned above, the characteristic three-band pattern of retinals is present in protonated Schiff bases,[19] although all are shifted to longer wavelengths. The bands have the same isomer dependence as the corresponding aldehydes, suggesting that they are due to the same electronic states. Visual pigments and bacteriorhodopsin both have protonated Schiff bases as chromophores; their main band absorbs at somewhat longer wavelengths than protonated Schiff bases in solution, owing most likely to electrostatic interactions between the chromophore and the protein.[28] The second transition (β band) of rhodopsin (which has an 11-cis chromophore) is at ca. 340 nm; this band loses intensity upon isomerization and has been assigned as the cis peak.[29] The energy separation between it and the main band is in the range expected for polyenes of the same length. There is no vibrational structure in this band, even at 77 K. No γ band is clearly resolved in rhodopsin but there is some evidence for a chromophore absorption band under the 290-nm peak of the pigment[28] which is due primarily to the aromatic amino acids of the opsin. This is in the wavelength range that a γ band would be expected for this molecule.

In light-adapted bacteriorhodopsin, the main absorption band appears at 570 nm and there is a weak subsidiary band at 410 nm. Since this band is structured and separated by about 7000 cm^{-1} from the main band, it is apparently a cis band whose low oscillator strength is due to the presence of an all-trans chromophore. No γ band is detected in the absorption spectrum but a clear CD band at about 330 nm is evident. In contrast, dark-adapted bR has an increased absorbance at 330 nm[30] which appears to be due to the presence of its 13-cis chromophore. Based on its energy separation of about 13 000 cm^{-1} from the main band and increased oscillator strength for the 13-cis isomer it would appear to correspond closely to the γ band.

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