Theoretical Studies of the Visual Chromophore

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The past few years have seen a renewed effort by physical chemists to understand the structure and electronic properties of the retinal isomers. Interest in these molecules has been stimulated by their importance in the visual process. The compound 11-*cis*-retinal is the chromophore of the visual pigment rhodopsin, absorption of light by which appears to be the initial step in a series of processes that finally result in the generation of a nerve impulse.[1] The retinal isomers are linear polyene aldehydes, many of whose physical properties are determined primarily by their delocalized π electrons. Since molecular quantum mechanics has been very successful in treating π-electron systems,[2-6] the visual pigments provide a rare opportunity for theory to aid in the understanding of a molecule essential to a biological process. Moreover, the retinal isomers have proved to be interesting in their own right, and attempts to fully understand their properties have stimulated theoretical, spectroscopic, and photochemical investigations that bear on a range of problems.[7]

This Account is devoted primarily to a discussion of the conformational and spectroscopic properties of the retinal isomers, with emphasis on 11-*cis*-retinal. However, it must be realized that even a complete characterization of these molecules represents only a first step in the understanding of the visual pigment, in which 11-*cis*-retinal is covalently bound to the protein opsin by a protonated Schiff base linkage with a lysine.[8] Little is known of the details of the lipoprotein environment of the chromophore in the pigment. The large spectral shift observed in going from an isolated chromophore to the pigment suggests that there are significant changes in the electronic structure, whose exact origins are still far from certain. It is clear, nevertheless, that recent advances in our understanding of the ground- and excited-state properties of the retinal isomers provide a firmer foundation for the discussion of the spectroscopic properties of the visual pigment. This is particularly true at the present time when a variety of techniques, such as resonance Raman,[9-11] circular dichroism,[12-21] and picosecond laser[22] studies, are being applied to unbleached rhodopsin and the various intermediates that occur in the visual cycle and when artificial pigments formed from synthetic analogs of retinal and opsin are being used to probe the nature of the chromophore–protein interaction.[23-26]

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Known Properties of the Retinal Isomers

Early theoretical studies on the conformation of isoprenic polyenes such as retinals were stimulated by the efforts of Zechmeister and coworkers to isolate and characterize carotene pigments.[27] For these molecules, the all-trans conformation of the polyene chain is most stable since any cis linkage along the chain introduces some steric hindrance. For example, in 9-*cis*-retinal (Figure 1c) the proximity of 8-H and 11-H causes a small increase in conformational energy.[28] However, the steric hindrance in this isomer is much smaller than that in 11-*cis*-retinal, the prototype of a hindered *cis*-polyene. In its planar conformations, 12-s-trans (Figure 1d) and 12-s-cis (Figure 1e), the nonbonded repulsion between 10-H and 13-CH_3 or between 10-H and 14-H would result in a very large destabilization so that the molecule must twist about one or more bonds to reduce this interaction.[28] Consequently, the detail of the conformation in the strained region (10-C–13-C) has been a question of great interest. Pauling estimated the destabilization of the π electrons due to the deviations from planarity and prediced that hindered *cis*-carotenes would not be present in an equilibrium mixture at room temperature.[29,30] Although the effects considered by Pauling clearly contribute, his estimates of their magnitude were too high. In fact, the ground-state conformation of 11-*cis*-retinal has been shown experimentally to have a free energy only 1.5 kcal greater than that of the all-trans isomer.[28,31]

Considerable attention has been given also to the orientation of the β-ionone ring with respect to the chain in retinal and other carotenoids.[28] There are two planar ring–chain orientations, s-trans (Figure 1b) and s-cis (Figure 1a). Nonbonded repulsions between the methyl groups in the ring and the nearest hydrogens (7-H and 8-H) of the chain raise the energy of both planar structures, so that significant distortions are expected; most important is twisting about the 6-C–7-C bond, which leads to dihedral angles $\phi_{6\text{-}7}$ different from 0° (s-cis) or 180° (s-trans). The preponderance of twisted s-cis structures in all the carotenoids whose crystal structure has been determined suggests that this is the preferred conformation.[28] However, the range of angles that has been observed, 35–80°, indicates that packing forces and the structure of the rest of the molecules are factors that also contribute to determining the value of $\phi_{6\text{-}7}$. Moreover, there appear to exist two crystal forms for many of the retinal and carotenoid-related species.[32] The two forms might well be a consequence of having the ring distorted s-cis in one and s-trans in the other. This is the case for retinoic acid,[33] but additional structure determinations are needed for other systems.

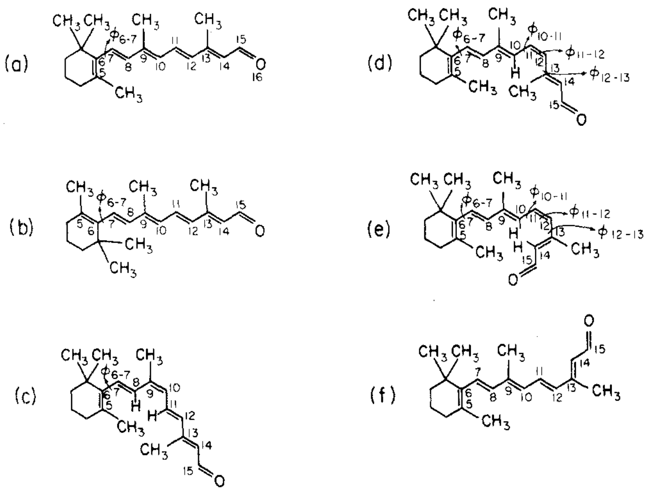

Figure 1. Isomers and conformers of retinal: (a) all-trans,6-s-cis; (b) all-trans,6-s-trans; (c) 9-cis; (d) 11-cis,12-s-trans; (e) 11-cis,12-s-cis; (f) all-trans,12-s-cis. Some important dihedral angles are indicated in the figure.

The spectroscopic properties of the retinal isomers have been studied by many workers,[28] but are far from being completely understood. For the 11-cis isomer, in particular, there are a number of spectral anomalies related to the distortion of the polyene chain. All of the isomers have an intense band at 360–380 nm and additional absorption in the region 300 and 240 nm. The latter has been interpreted as being due to two or three weaker bands, of which one at 250 nm is most clearly visible in certain isomers (*e.g.*, 11-cis and 13-cis). The spectra are generally diffuse, though there appears to be some vibrational structure at low temperatures in certain of the shorter wavelength absorptions.[34-36] The absorption maximum (λ_{max}) of 11-*cis*-retinal ($\lambda_{max} \simeq 370$ nm) in EPA at 300 K appears at only slightly shorter wavelengths than that of all-trans ($\lambda_{max} \simeq 375$ nm) and is to the red of the planar cis isomers (9-cis, $\lambda_{max} \simeq 366$ nm; 13-cis, $\lambda_{max} \simeq 369$ nm).[35] Most other molecules with sterically hindered cis linkages exhibit much greater shifts to shorter wavelengths in λ_{max}. This is consistent with the expected behavior of π-electron systems that are twisted about "essential" single bonds[2] (*i.e.*, the bonds that have small partial double-bond character in the ground state of the polyene). Thus, the position of the λ_{max} of 11-*cis*-retinal must be reconciled with the fact that the polyene chain of the molecule is significantly distorted from planarity.

A related observation is the large temperature dependence of the main absorption band of 11-*cis*-retinal. As was shown by Jurkowitz, *et al.*,[37] upon cooling from room temperature to 77 K *all-trans*-retinal, as well as the "unhindered" 9-cis and 13-cis isomers, exhibit a shift in λ_{max} of about 14 nm to longer wavelengths, and their maximum extinction coefficient (ϵ_{max}) is increased by about 11%. Although 11-*cis*-retinal exhibits a comparable shift in λ_{max}, its extinction coefficient rises dramatically as a function of temperature; it has increased by 60% at 77 K rela-

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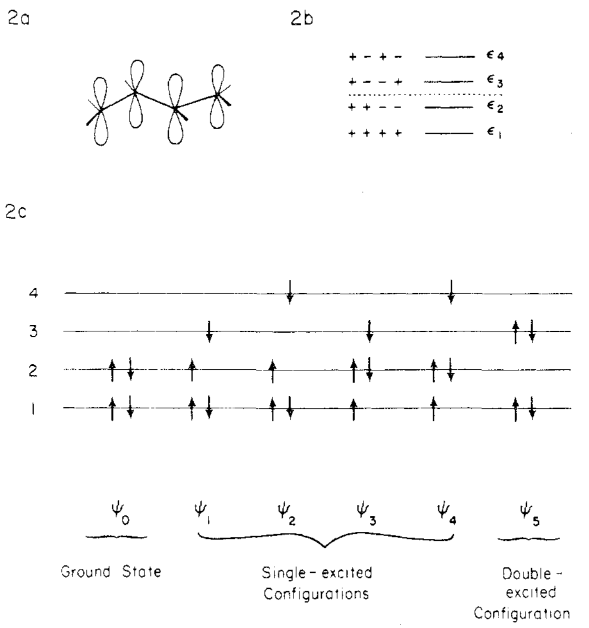

Figure 2. Butadiene: (a) schematic drawing of the four π orbitals; (b) one-electron energy levels and wave functions; (c) electronic configurations.

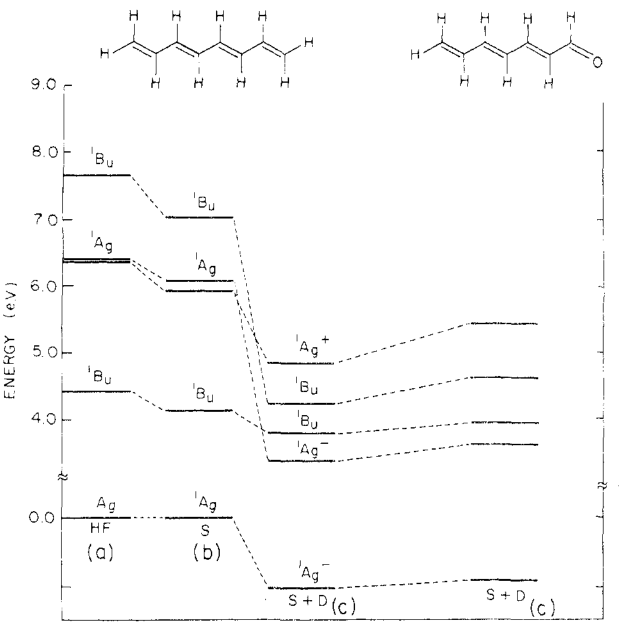

Figure 3. Ground-state and four lowest excited states of octatetraene and of the corresponding aldehyde: (a) HF, Hartree-Fock energies; (b) S, energies obtained using only the single excited configurations; (c) S + D, energies obtained using both single and double excited configurations.

tive to its room-temperature value. More detailed measurements which confirm the work of Jurkowitz, *et al.*, and include some of the higher transitions have been reported by Becker, *et al.*,[36] and by Sperling and Rafferty.[34,35]

General Features of Polyene Excited States

A complete characterization of the spectroscopic properties of retinal isomers requires an understanding of the nature of their various states, including a knowledge of the potential-energy surfaces for the ground and excited states. The accurate calculation of potential-energy surfaces is particularly important for sterically hindered polyenes because the effective transition energies and magnitude of the Franck–Condon factors are expected to be sensitive functions of twisting about various bonds.

The interpretation of the spectra of linear polyenes was one of the early successes of π-electron theory. Most of the observed transitions correspond to the excitation of bonding π electrons to antibonding π^* orbitals. To clarify the nature of the electronic transitions, we consider as an example the butadiene molecule which is the simplest polyene. It can be considered as a four-π-electron problem with the nuclei and σ electrons playing the role of a fixed core. There are four atomic orbitals (Figure 2a) which can be combined to form four molecular orbitals. The signs of the atomic orbital coefficients (all equal approximately to 0.5 in magnitude in the zero overlap approximation) and the relative energies of the four molecular orbitals are shown schematically in Figure 2b; orbitals 1 and 2 are bonding orbitals and 3 and 4 are antibonding orbitals. The ground-state configuration (ψ_0) and the four possible single excited configurations ($\psi_1,\psi_2,\psi_3,\psi_4$) are shown in Figure 2c; the arrows indicate the spin pairing corresponding to the singlet configurations being considered here. From these four excited configurations, which are the only ones considered in the usual Hückel and Pariser–Parr–Pople (PPP) type approximations to molecular orbital theory,[2-6,38] it is possible to form four excited singlet states (1^1B_u, $1^1A_g^+$, $2^1A_g^-$, 2^1B_u). The PPP energies for the four corresponding states of octatetraene are given in Figure 3; the butadiene levels have the same structure though their energies are, of course, considerably higher. These states have dominant contributions from the listed configurations (1^1B_u, ψ_1; $1^1A_g^+$, $\psi_2 + \psi_3$; $2^1A_g^-$, $\psi_2 - \psi_3$; 2^1B_u, ψ_4). The 1^1B_u state is strongly allowed and corresponds to the intense lowest energy band normally observed in polyenes.[2-6] The two ^1A_g states are seen to be nearly degenerate and correspond to forbidden transitions from the ground state, which is also totally symmetric ($1^1A_g^-$). The upper 2^1B_u state is weakly allowed and has also been observed in some all-trans polyenes.

One of the two ^1A_g states, the $^1A_g^+$ state,[2,27] becomes allowed in configurations of the polyene chain involving cis double bonds or s-cis single bonds that destroy the inversion symmetry of the molecule. It gives rise to the so-called "cis peak." The intensity of the cis peak in carotenes with cis double bonds was studied extensively by Zechmeister and his colleagues, and it was found that this band increases in intensity as the cis double bond becomes more central in the molecule.[27] The cis peak was observed to be intermediate in energy between the two lowest ^1B states as predicted by PPP calculations. Since *s-cis*-butadiene appears to be significantly less stable than the s-trans form, its spectroscopic properties can only be deduced from molecules in which the two double bonds are part of the same ring as in cyclohexadiene.[5] The cis peak in such molecules is particularly intense. Also of interest is the fact that the main ^1B band of s-cis dienes appear at markedly longer wavelengths and lower intensities than those of s-trans dienes.[5]

Since the $2^1A_g^-$ state remains forbidden even in the cis isomers of polyenes, its position has been difficult to determine. As shown in Figure 3, PPP cal-

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culations with single excited configurations result in its energy being close to the $^1A_g{}^+$ state. However, recently, a weakly absorbing state has been detected by Hudson and Kohler below the main 1^1B_u band in a number of high-resolution spectra of polyenes.[39,40] Based on the anomolously long radiative lifetimes observed for many polyenes and the different solvent shifts in absorption and emission, they have argued that this state is likely to be the lowest excited state. It was assigned as the $^1A_g{}^-$ state on the basis of PPP calculations in which all double excited configurations were included in addition to the single excited states.[41] The most important such configuration is ψ_5, shown in Figure 2c. The primary effect of the double excitations, other than a lowering of the ground state, was found to be the lowering of the transition energy of the $2^1A_g{}^-$ state below that of the main 1^1B_u transition (see Figure 3). *A priori* calculations also reproduce this ordering of energy levels.[42] The presence of the low-lying $2^1A_g{}^-$ state is predicted at a simpler level of approximation if either a valence bond or exciton model is applied to polyenes;[41] in butadiene, the main contribution to the $2^1A_g{}^-$ state comes from the so-called "long-bonded" valence-bond structure. The relative energies of the $2^1A_g{}^-$ and the 1^1B_u state, as well as other of the higher "covalent" (^1A_g) and "ionic" (^1B_u) states, depend on the range of the Coulombic electron-electron repulsion in the polyenes.[43] For a short-range repulsive interaction, the covalent states with appropriate valence-bond spin pairing are low-lying, while for a long-range interaction, the ionic states best represented by the molecular orbital approximation can be lowest. The "chemical range" of the Coulombic repulsion is such that the 1^1B_u and $2^1A_g{}^-$ states are very close in energy, and it is difficult to show theoretically which one is lowest. However, as pointed out above, the experimental studies of certain polyenes and diphenylpolyenes suggest that the 2^1Ag^- state is below the ^1B_u state.[40] Final verification of this fact will depend on further experimental studies. Certain workers[44] have recently been unable to find the low-lying $2^1A_g{}^-$ in low-resolution spectra of gaseous hexatriene; however, as they themselves were careful to point out, the conditions were such that not finding the state is no evidence against its being there.

Recent Developments

In this section we describe some recently developed theoretical methods for polyenes and discuss the results obtained in their application to the retinal isomers.

Calculation of Potential-Energy Surfaces. A detailed interpretation of the electronic transitions and photochemical processes in the retinal isomers requires a knowledge of ground- and excited-state potential surfaces. Completely empirical treatments in which the energy surface is expressed as a function of potential parameters fitted to the available information (*e.g.*, equilibrium geometry, vibrational frequencies) have had considerable success in applications to molecules for which a localized electron description is applicable.[45] For conjugated molecules, however, the importance of delocalization introduces difficulties into such an empirical treatment, particularly if one wishes to treat excited states. A method that is appropriate assumes separability between the σ and π electrons and treats the σ electrons *via* empirical potential functions and the π electrons by a semiempirical approach, such as Hückel or PPP theory. In this approximation, the total potential energy is expressed as a function of the vector **r**, which represents the coordinates of all the atoms, in the form

$$V^{\mathrm{N}}(\mathbf{r}) = V_\sigma(\mathbf{r}) + V_\pi{}^0(\mathbf{r}) + \Delta V_\pi{}^{\mathrm{N}}(\mathbf{r}) \tag{1}$$

where $V_\sigma + V_\pi{}^0$ is the sum of the σ- and π-electron energies for the ground state and $\Delta V_\pi{}^{\mathrm{N}}$ is the π-electron excitation energy. Most of the work done to date has made additional simplifying assumptions about the σ framework. For example, in the calculation of torsional potentials, bond lengths and bond angles have been assumed fixed and the potential energy is approximated by

$$V^{\mathrm{N}}(\mathbf{r}) = V^{\mathrm{nb}}(\mathbf{r}) + V_\pi{}^0(\mathbf{r}) + \Delta V_\pi{}^{\mathrm{N}}(\mathbf{r}) \tag{2}$$

where $V^{\mathrm{nb}}(\mathbf{r})$ is the σ-electron energy contribution of nonbonded interactions, which are the only interactions that vary under the rigid geometry restriction. This type of approximation has been used in predicting the ground-state torsional angles of sterically hindered molecules, such as biphenyl. In other applications, the bond lengths have been allowed to vary through the use of a bond-length, bond-order relationship. Corresponding calculations have been made for excitation energies, but these have generally required large changes in the basic parameters to obtain agreement with experiment. Furthermore, changes in geometry in the excited states have been generally ignored. While qualitatively correct behavior is predicted,[2,6] quantitative results are difficult to obtain by such simplified methods.

Recently a unified semiempirical approach to the ground- and excited-state potential surfaces of conjugated molecules has been introduced.[46] A single parameter set is used to obtain the properties considered, and all geometric degrees of freedom (bond lengths, bond angles, and torsional angles) are included in the calculation. To make possible a rapid determination of the potential surfaces with simultaneous variation of all coordinates, the total potential energy (eq 1) is approximated by an analytic function of the Cartesian coordinates **r**. The function $V_\sigma(\mathbf{r})$ is given by an empirical expression of the type used in potential-surface calculations for saturated hydrocarbons. The π-electron contribution to the energy ($V_\pi{}^0(\mathbf{r}) + \Delta V_\pi{}^{\mathrm{N}}(\mathbf{r})$) is obtained from an SCF-LCAO-MO-CI method of the PPP type corrected for nearest-neighbor overlap; the latter correction is essential for obtaining correct excited-state poten-

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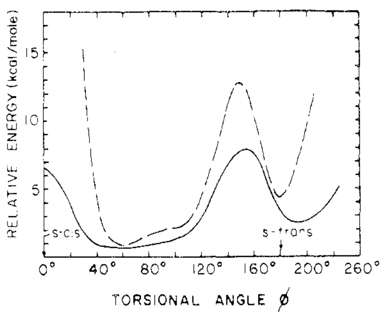

Figure 4. Torsional potential for the cyclohexene ring in retinal: (- - -) calculated from eq 2 with fixed bond lengths and bond angles; (—) calculated from eq 1 including relaxation of bond lengths and bond angles.

tials.[46] Parameters for the σ potential function and π-electron integrals were determined by a simultaneous fit to a wide range of experimental data for ethylene, butadiene, benzene, and propylene. The approximate analytic expression for the potential allows one to apply efficient energy-variation techniques to evaluate the minimum-energy geometry in the multidimensional space corresponding to a molecule as complicated as retinal. At the calculated minimum, the vibrational frequencies can be determined from the analytic second derivative of the molecular potential.

The application of this technique to the ground state of butadiene and other polyenes has led to the satisfactory evaluation of the stable conformations, vibrational frequencies, and torsional barriers.[46] Changes in geometry upon excitation have also been predicted with reasonable accuracy. In the polyenes studied, it was found that single bonds become shorter and double bonds stretch in the first allowed excited (1^1B_u) state. There is also a significant change in the double-bond stretching frequencies. These results can be correlated with the Franck–Condon factors and vibrational bands observed in the electronic spectrum.[47]

Applications to Retinals. We now consider results obtained for the retinal isomers and compare them with experimental evidence where available.

Ground-State Conformation. For the ground-state conformation of the retinals, the two questions of primary interest concern the orientation of the cyclohexene ring with respect to the polyene chain in all the isomers and the nature of the chain distortion in the 11-cis isomer.

Calculated results for the torsional potential of the cyclohexene ring for rotation with respect to the dihedral angle ϕ_{6-7} (Figure 1) are shown in Figure 4. The solid line was obtained from eq 1 with full minimization with respect to all degrees of freedom except for the torsional angle under study, which is chosen as the "adiabatic" variable.[46,48] Previous studies of the torsional potentials have not included the possible relaxation of steric repulsion by bond stretching and bond angle bending. They lead to a qualitatively similar potential form that differs in having the high-energy regions at still higher energies. The results of such a calculation that uses eq 2 are given as the dashed line in Figure 4.[49] There are also shifts in the position of the minima [*e.g.*, that at "s-trans"] due to a difference in the symmetry of the molecule in a rigid rotation *vs.* adiabatic energy calculation.

Crystallographic evidence has shown that a twisted s-cis conformation exists in *all-trans-* and 11-*cis*-retinal.[50-52] The angles found in the crystals are in very good agreement with the calculated values,[53] that is, the all-trans results are −58.3 (crystal) and −50° (calcd) while those for 11-cis are −41.4 (crystal) and −44° (calcd). It is interesting that the calculations predict the direction of change between *all-trans-* and 11-*cis*-retinal. This is a consequence of the change in the π bond order of the 6–7 bond that results from the distortion of the sterically hindered polyene chain in the 11-cis compound relative to the planar chain in the all-trans molecule.

Since the possibility of significant changes in geometry exists in crystals due to packing interactions, particularly for shallow potentials such as that found for the s-cis region of ϕ_{6-7} (Figure 4), it is important to have independent data for the molecule in solution. A study of the solution conformation of the cyclohexene ring in β-ionone and 11-*cis-* and *all-trans*-retinals was first made by nuclear magnetic resonance (NMR) methods.[49] Long-range coupling constants and nuclear Overhauser enhancements (NOE) were determined. Of primary interest are the results for protons 7-H and 8-H (see Figure 1), which permit one to determine the orientation of the ring relative to the chain.

For a π-electron system, the coupling between two protons with five intervening bonds are usually dominated by the π-contribution, such that[54,55]

$$\begin{aligned} J_{\mathrm{HH}'}(\mathrm{cis}) &\simeq A \sin^2 \phi \sin^2 \phi' + B_{\mathrm{c}} \\ J_{\mathrm{HH}'}\ (\mathrm{trans}) &= A \sin^2 \phi \sin^2 \phi' + B_{\mathrm{t}} \end{aligned} \qquad (3)$$

where $A \simeq$ 5–8 Hz and B_c, B_t are small constants ($|B_i| \leq 1$ Hz). As can be seen from the labeling in Figures 1a and 1b, the coupling of 7-H with the methylene group protons 4,4′-CH_2 and the methyl group protons 5-CH_3 should be governed by eq 3 with ϕ identified as the ring torsion angle ϕ_{6-7}; the angle ϕ' is the approximately fixed or average torsional angle of the hydrogens in 4,4′-CH_2 or 5-CH_3 relative to the 4-C–5-C–6-C plane or the 5-CH_3–5-C–6-C plane, respectively. From eq 3 and the measured coupling constants, a value of 28 to 36° is obtained for the magnitude of the angle ϕ_{6-7}. While the coupling constants demonstrate the out-of-plane twisting of the ring, they cannot (as seen from the form of eq 3) distinguish between an s-cis (±28 to ±36°) and an s-trans (±146 to ±152°) conformation. Looking at the calculated torsional potential in Figure 4, we see that a large out-of-plane angle is predicted for the s-cis conformation while the s-trans conformation is predicted to be planar or near planar. Consequently, the observed magnitude of the

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coupling constants appears to rule out the s-trans conformation. The fact that the coupling constants increase with temperature confirms that the primary form is s-cis.[49]

Additional evidence on the ring orientation is obtained from the nuclear Overhauser effect measurements.[49] Low-temperature (−50°) results show that protons of 5-CH_3 interact strongly with 8-H, and those of 1,1′-CH_3 with 7-H. This result clearly excludes the s-trans conformation in which the opposite result would be predicted since the relative NOE values are related to the inverse sixth power of the distance.[49,56] As the temperature is raised the NOE's between 7-H,8-H, and 5-CH_3,1,1′-CH_3 tend to equalize, suggesting that either the cyclohexene ring in the s-cis isomer is rotating through a larger range of angles ($25° \leq |\phi_{6-7}| \leq 90°$) or that the s-trans conformation is also contributing to the equilibrium distribution. The former possibility seems the more likely one because of the temperature dependence of the coupling constants. The NMR results on the ring–chain orientation are thus consistent with the theoretical torsional potential for ϕ_{6-7} shown in Figure 4. Also, the variation of the ring–chain orientation found in crystals for different molecules of the retinal type[28] is in agreement with the very broad s-cis minimum.

For 11-*cis*-retinal (Figures 1d and 1e), in addition to the ring–chain torsional angle there is the conformational problem of the distortions in the 10-C-14-C region resulting from the presence of steric interactions that are absent in the all-trans compound and very small in the other cis isomers (*e.g.*, 9-cis, 13-cis). The torsional potential for the ground state of 11-*cis*-retinal as calculated from eq 2 is given in Figure 5.[57] The more general method making use of eq 1 and regarding ϕ_{12-13} as the "adiabatic variable" confirms the general features of the curve.[53] The most important result of the calculations is that, in addition to the distorted 11-cis,12-s-trans minimum ($\phi_{12-23} \simeq 120°$) that had been assumed to represent the structure in all earlier studies, there is a distorted 11-cis,12-s-cis minimum ($\phi_{12-13} \simeq 50°$). Further, as shown in the figure, the twisted 12-s-cis structure is calculated to be more stable than 12-s-trans, although the energy difference is small and probably within the inaccuracies of the method of calculation.

The "prediction" of a 12-s-cis geometry for 11-*cis*-retinal was confirmed by a subsequent X-ray analysis ($\phi_{12-13} \simeq 41°$).[50,52] (Theoretical chemists tend to use the word "prediction" rather loosely to refer to any calculation that agrees with experiment, even when the latter was done before the former; the 12-s-cis geometry was a prediction in the true meaning of the word.) It was also determined that, in the crystal, the dihedral angles around the 10-C-11-C single bond and 11-C-12-C double bond correspond to an essentially planar geometry. This disagrees somewhat with the calculations[53,57] which yield a twist of 8–10° for both dihedral angles. The importance of including degrees of freedom other than torsion in a theoretical treatment of the geometry is made clear by looking at some of the bond angles in the sterically hindered 10-C-13-C region:[53] *e.g.*, the 10-C-11-C-12-C angle, which is equal to 121° (calcd), 123° (exptl) in *all-trans*-retinal opens out to 126 (calcd), 128° (exptl) in 11-cis.

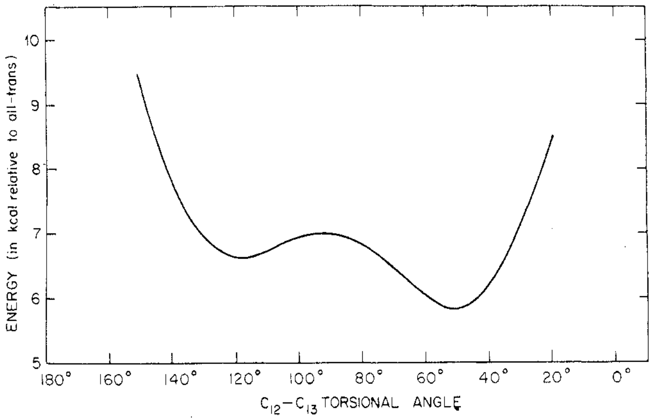

Figure 5. Torsional potential for 12-C-13-C bond in the ground state of 11-*cis*-retinal obtained from eq 2 with fixed bond lengths and bond angles.

An NMR study of the polyene chain conformation of *all-trans-* and 11-*cis*-retinals dissolved in deuterioacetone has been completed recently.[53] Spin–spin coupling constants and nuclear Overhauser interactions were used in a manner analogous to that described above in the determination of the cyclohexene ring orientation. The experiments indicate that there is a small twist about the 10-C-11-C bond and a larger one about the 12-C-13-C bond. The NOE measurements of 11-*cis*-retinal also show that 10-H is relaxed almost equally by 14-H and 13-CH_3. The most reasonable explanation of this result (see Figure 1) is that in deuterioacetone solution at room temperature 11-*cis*-retinal is present as an equilibrium mixture of the 12-s-cis and 12-s-trans conformers, with the two being present in approximately equal amounts. Low-temperature measurements suggest that the 12-s-trans isomer is slightly more stable. The apparent energy difference between the two isomers is only ~1 kcal and, therefore, could be sensitive to solvent effects and other perturbations. There is some evidence[58] that in a cyclohexane solution the equilibrium is shifted toward the 12-s-cis form. Dipole moment calculations suggest that 12-s-trans should be stabilized relative to 12-s-cis in a polar solvent.[59]

Excited-State Conformation and Spectra. The spectroscopic properties of the retinal isomers were briefly described above and the questions concerning them were outlined. Calculations concerned with the excited states of the retinal isomers can serve to answer some of these questions. In what follows, the discussion is restricted primarily to the lowest strongly absorbing π,π^* state of the retinal isomers. One of the striking characteristics of the absorption to this state from the ground state is that it is very broad and essentially diffuse. This is true of all the retinal isomers and many simpler related compounds, such as β-ionone. To investigate the origin of the diffuseness, Franck–Condon factors for the transition were calculated. This was done by obtaining the ground- and excited-state potential sur-

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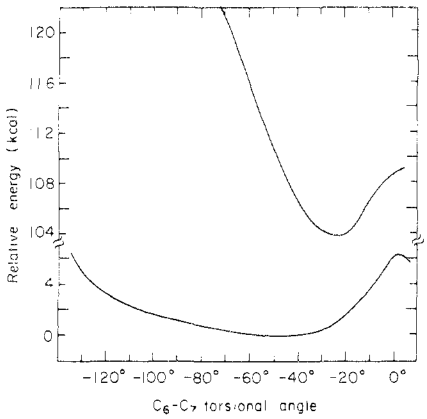

Figure 6. Torsional potential for the cyclohexene ring in retinal obtained from eq 1, including relaxation of bond lengths and bond angles: lower curve, ground electronic state; upper curve, π,π^* excited electronic state.

faces as described earlier in this Account, performing a complete vibrational analysis for the two surfaces, and then evaluating the required vibrational overlap integrals. The procedure for doing such a Franck–Condon factor calculation for a polyatomic molecule including all of the degrees of freedom has only recently been developed to the stage where often unjustified approximations are not necessary.[47] Results were obtained for the relative intensities of the vibronic transitions in the absorption spectrum of β-ionone and *all-trans-* and 11-*cis*-retinal.[47,48] As in most polyenes, the strongest vibrational progression is due to C–C stretching modes that arise from the change in bond lengths upon excitation. In addition to the major modes, a number of low-frequency torsional modes coupled with skeletal wagging vibrations are found to be strongly active. The contribution from the latter is due primarily to the difference in the torsional potential of the 6-C–7-C bond in the ground and excited state (Figure 6). As a consequence of the change in bond orders on excitation (the "single" bonds have increased bond order and that of the "double" bonds is decreased), the excited-state potential has its minimum shifted closer to the planar configuration and has a form that is considerably narrower and steeper than that of the ground state. Although there are many strongly active low-frequency modes as a result of this change in the torsional angle and they are expected to be unresolved at a width of 100–200 cm^{-1}, their contribution is calculated to be insufficient to "fill in" the C=C stretching progression; that is, instead of the completely diffuse experimental spectrum, one showing some broad peaks due to the C=C stretch is calculated.[48] One possible explanation of this disagreement is that the difference between the equilibrium torsional angle in the ground and excited state is larger than the calculated one. Model calculations in the complete vibrational space of the molecule show that an angular change of 12–15°, instead of the 8° resulting from the semiempirical potential, would be sufficient to produce a completely diffuse spectrum. Another factor that might play an important role is the very broad nature of the ground-state potential about the 6-C–7-C bond (Figure 6). Molecules in slightly varying local environments may have their minima over a range of different torsional angles. As a result, the vibronic structure would change significantly from site to site so as to yield a completely diffuse spectrum.[48] A third contributor to the diffuseness is the possibility that a second allowed excited electronic state is contributing to the intensity in the observed region. Such a state is calculated to exist in a treatment including doubly excited configurations; it is in fact the state corresponding to the low-lying ${}^1A_g{}^-$ state in butadiene, that is made allowed by the reduction in symmetry due to the presence of the aldehyde group in the retinals and β-ionone.[41,59]

Another suggested contributor to the diffuseness is the lowest n–π^* transition, which is expected to be in the same spectral region as the π,π^* band of the retinals. However, Christensen and Kohler[60] have shown that the lack of vibrational structure persists in molecules such as axerophthene that have no carbonyl group. Removal of the sterically hindered cyclohexene ring leads to the appearance of vibrational structure, in agreement with the above discussion.

For the 11-cis isomer of retinal, the maximum in the main absorption peak ($\lambda_{\max}$) is very similar to that of the unhindered isomers. Hubbard and Wald[28] pointed out that this was inconsistent with the expected nonplanar conformation, since twisting about essential single bonds induces blue shifts in the spectrum. The NMR solution studies and the crystal structure confirm, as pointed out above, that the 11-cis isomer is dominantly twisted about the 12-C–13-C single bond. A partial resolution of this paradox is based on the observation[57] that the main absorption peak of s-cis structures,[5,61,62] such as s-cis dienes, is shifted to longer wavelengths. Thus, a distorted 11-*cis*,12-s-*cis*-retinal could have its maximum close to that of the all-trans isomer as a consequence of the balance of two effects that act in the opposite direction. This hypothesis was tested by calculating the π,π^* excitation energies as a function of twisting about ϕ_{12-13}. Both s-cis and s-trans structures were included in the study.[57] It was found that there is indeed a balance between the "s-cis redshift" and the blue shift due to twisting about 12-C–13-C. At a torsional angle of 40°, 11-*cis*-retinal is predicted to have the same absorption maximum as the all-trans isomer. This value is very close to the calculated s-cis energy minimum, as well as to the crystal structure value of 41°. If the true solution situation at room temperature is an equilibrium of approximately equal populations of 12-s-cis and 12-s-trans, as suggested by the NMR studies,[53] the observed broad absorption peak would be the appropriate average of the absorptions of the two species.

Of interest in relation to these results are the properties of the non-naturally-occurring 14-CH_3 retinal that has been synthesized recently.[63] From Figure 1, it is clear that the addition of this methyl group should have little effect on most retinal isomers, other than the small spectral shift associated with such a substitution. In particular, the 14-CH_3 group introduces no additional steric hindrance in

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the s-trans conformation of the 11-cis isomer. However, steric hindrance between the 14-CH_3 and 10-H should greatly destabilize the s-cis conformation of 14-methyl-11-cis-retinal. As expected, the λ_{max} values of *all-trans-* and 14-methyl-9-*cis*-retinal are shifted slightly to the red relative to their respective retinal analogs.[63] However, the λ_{max} of the 11-cis isomer is shifted by approximately 25 nm to shorter wavelength. This shift in the spectrum can be explained in terms of a decreased contribution of the 12-s-cis form or the formation of a more strongly twisted 12-s-cis structure due to 10-H–14-CH_3 repulsion. In either case the results suggest that the 12-s-cis conformer is present in significant amounts at room temperature.

As to the anomalous temperature dependence of the 11-cis absorption spectrum relative to the other isomers, several suggestions follow from the theoretical results. The calculations for 11-*cis*,12-*s*-*cis*- and 11-*cis*,12-*s*-*trans*-retinals show that the former absorbs at longer wavelength while the latter has a larger oscillator strength. Thus, a temperature-dependent equilibrium between the two forms with 11-cis,12-s-trans more stable, as suggested by the NMR results,[53] would result in an increased oscillator strength (in agreement with experiment) and a shift of λ_{max} to shorter wavelength (in disagreement with experiment) as the temperature is lowered. It has been shown that the presence of the second (^1A_g-like) state in the same region may reduce the λ_{max} shift leading to better agreement with experiment.[59] Another contribution to the temperature dependence is related to the 12-C–13-C torsional potential and its qualitative correspondence to that for the cyclohexene ring orientation discussed above. A change in the population of vibrational energy levels of the ground state as a function of temperature could result in a significant shift due to variations in the Franck–Condon overlap integrals. Model calculations[48] have shown that a change in about 5° in the average value of $\phi_{12\text{-}13}$ in the ground state can reproduce the observed thermal modification of the main band in going from 70 K to 300 K. Thus, it is possible that a vibronic redistribution of intensities, as well as the change in electronic contributions due to conformational equilibria, are important for the unusual temperature dependence observed in 11-*cis*-retinal.

The Visual Pigments

While the conformational and spectral properties of the retinal isomers are coming to be well understood, knowledge of the visual pigments, which are composed of the chromophore 11-*cis*-retinal and a protein opsin, is in a far more primitive state. A central problem remains the interpretation of the large spectral shifts that occur on pigment formation and the considerable variation in the absorption maxima of pigments differing only in the nature of the protein to which the same chromophore is bound. Further, for any given pigment, the intermediates which appear in the visual cycle have a wide range of spectral maxima. Thus, a variation of the main absorption between 345 nm (3.60 eV) and 640 nm (1.94 eV) has to be accounted for by environmental effects. To understand these completely, it is clearly essential to have a knowledge of the structural details of the binding site of the chromophore and of the variation of the binding site in different proteins. At present, it is known that in rhodopsin retinal is bound to a lysine[8] of the protein by a protonated Schiff base linkage; the clearest evidence for protonation comes from resonance Raman studies including deuteration.[11] Otherwise there is almost no direct information about the chromophore environment in the naturally occurring lipoprotein system.

Experimental studies of protonated Schiff bases of the retinal isomers show that the absorption maximum shifts from 380 nm to ~450 nm under normal solvent conditions. Special solvents (*e.g.*, *o*-dichlorobenzene)[64,65] can give an additional shift to ~500 nm, but it seems unlikely that the latter has direct relevance to the mechanism of the shift in rhodopsin. A number of theoretical studies of the spectrum of retinal in the unprotonated and protonated form have been made.[66-70] It is clear from these calculations that the unprotonated Schiff base has an absorption maximum similar to that of retinal itself and that protonation results in a shift to longer wavelength. The exact magnitude of the shift is unfortunately difficult to determine in an unequivocal fashion because of the problems raised by the presence of the additional charge; *i.e.*, it is difficult to be sure of the appropriate parameters for the semiempirical calculation and it is difficult to account for the effect of the solvent in a quantitative fashion. Nevertheless, analyses[69,70] of the available calculations suggest that protonation *per se* is unlikely to shift the absorption significantly beyond that observed for the protonated Schiff base in solution. Thus, additional contributions are required to obtain the spectral shift observed in rhodopsin and its analogs. Calculations show that, due to the large difference between the molecular charge distribution of the ground and the excited state of the protonated Schiff base of retinal, external charges at reasonable distances (*e.g.*, those from a $-CO_2^-$ or $-NH_3^+$ group of the protein) can give shifts of the observed magnitude.[69,70] Either positive or negative charges can produce shifts to longer or shorter wavelengths depending on their position relative to the chain; *e.g.*, if a carbon atom is more negative in the ground than in the excited state, a positive charge near that atom would stabilize the former more than the latter and give a shift to shorter wavelength. Another theoretically possible mechanism for introducing the observed shifts is by twisting of the chromophore. Calculations[48] show that the polyene chain of retinal is quite flexible. Twists about double bonds induced by the protein and requiring only a few kilocalories could yield the required shifts to longer wavelengths.[70] Corresponding calculations suggest that

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medium polarizability effects of the type expected from aromatic amino acids and various lipids are unlikely to be large enough to produce the observed shifts.[71]

From the above discussion, which has been kept qualitative on purpose, it should be clear that theory can only suggest what aspects of the system could account for the observed spectral properties of the visual chromophore. The theoretical results cannot say which mechanism or combination of mechanisms is the one that actually occurs in nature. For progress in answering that question, additional experimental information concerning the details of the chromophore environment is required.

One approach to the chromophore–protein interaction makes use of the circular dichroism (CD). Although no CD is observed for retinal in solution (where the different enantiomers are rapidly interconverting and present in equal amounts), all visual pigments exhibit strong optical activity.[12-21] The absolute and relative intensities of the two CD bands that can be attributed to the chromophore are found to vary considerably in different environments (rod outer segments or a particular detergent) and when 11-*cis*-retinal is replaced by another isomer or by some synthetic analog.[26,72] Two hypotheses have been proposed to explain the optical activity of the visual pigments. One model suggests that the chromophore transition is coupled to an asymmetric center in the protein, while the second is based on the idea that the protein binding site selects a unique asymmetric conformation of the retinal Schiff base. Calculations indicate that either model is capable of accounting for the large rotational strengths observed for the visual pigments.[73] Detailed studies of the dependence of the CD spectra on the specific nature of the chromophore through the use of artificial pigments may eventually permit a choice to be made between the two models and give additional information about the nature of the interactions involved.

In addition to interpreting the spectra of the unbleached visual pigments, it is of considerable interest to understand, on a molecular level, the events that take place following light absorption. It is usually assumed that the initial step in visual excitation is the photoisomerization of the 11–12 double bond of the chromophore.[1] However, the details of this process and the subsequent steps in the bleaching sequence are not understood. A number of experiments indicate that rhodopsin and the first two intermediates of bleaching involve alternate states of the chromophore since no changes have been detected in the opsin before the transition to metarhodopsin I.[7,15,16,74,75] It has been suggested that the presence of three distinct steps that involve the retinal alone can be understood if the chromophore is bound in the 12-s-cis conformation.[57] Thus, the series of reactions

$$\text{rhodopsin} \xrightarrow{h\nu} \text{prelumirhodopsin} \xrightarrow{\text{dark}} \text{lumirhodopsin}$$

could be associated with the transformations

$$\text{11-cis,12-s-cis} \xrightarrow{h\nu} \text{11-trans,12-s-cis} \xrightarrow{\text{dark}} \text{all-trans}$$

Such a set of intermediates is consistent with the distribution of double bond character in the ground and excited states and with the spectral shifts associated with the intermediates. However, there is no direct evidence concerning the conformation of the native chromophore nor those of these intermediates.

It will not be possible to evaluate models that describe changes in the chromophore before the basic nature of the chromophore–opsin interaction is known. Moreover, it will be necessary to characterize the various steps in bleaching with more sensitive probes than spectra alone can provide. Several important results that have recently appeared should be mentioned in this regard. The first is the demonstration that the primary photoproduct, prelumirhodopsin, is formed within 6 psec.[22] The second is the recent demonstration that the transition dipole of the chromophore in the intermediates of bleaching remains nearly parallel to that of rhodopsin.[76] The third is the resonance Raman observation of photostationary states involving several of the intermediates of the visual cycle.[11] The interpretation of these studies, as well as others that hopefully will be made in the near future, should provide information essential for an understanding of visual excitation on a molecular level.

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