

Chapter 8

The Electronic States of Carotenoids

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Summary

The photochemistry of carotenoids is determined by the electronic structures of their low energy, excited states. This chapter first relates the optical spectroscopy of carotenoids to current theoretical descriptions of polyene electronic states. Highly detailed spectroscopic information now available for model polyenes leads to a simple, three-level energy scheme: $S_2(1^1B_u) > S_1(2^1A_g) > S_0(1^1A_g)$. Optical studies of polyenes and carotenoids of intermediate length demonstrate that increases in conjugation lengths invariably result in $S_2 \rightarrow S_0$ emissions replacing the characteristic $S_1 \rightarrow S_0$ emissions of short polyenes. This cross-over to S_2 fluorescence can be accounted for by the energy gap law: decreases in S_1 energies lead to sharp increases in the rates of $S_1 \rightarrow S_0$ nonradiative decay with increasing conjugation. The dominance of S_2 emissions in long polyenes means that the S_1 states of the carotenoids employed by photosynthetic systems are difficult to locate using fluorescence detection. Even less information is available regarding the energies of carotenoid T_1 states due, in large part, to the absence of confirmed phosphorescence in any polyene or carotenoid. This chapter critically examines the use of extrapolation procedures, including applications of the energy gap law, in estimating the S_1 and T_1 energies of long, non-emissive carotenoids. In spite of the inherent limitations of extrapolations, estimates of S_1 energies in molecules such as spheroidene and β -carotene now are adequate for understanding the

mechanisms of singlet energy transfer in photosynthetic systems. Outstanding issues still to be addressed include: the development of a more quantitative understanding of the effects of conjugation, substitution, isomeric structure, and solvent environment on the energies and dynamics of S_1 and S_2 states; the extension of the limited data base of T_1 energies to longer polyenes and carotenoids; the evaluation of the effects of conformational disorder on carotenoid spectroscopy and photochemistry; and the consideration of additional low-lying singlet states in the energy level diagram.

I. Introduction: Low Lying Excited Singlet and Triplet States in Carotenoids

The roles of carotenoids as light-harvesting and photoprotective pigments and their use as visual chromophores ultimately are determined by the energies, structures, and dynamics of their low-energy, excited electronic states. An accurate description of these states thus is the crucial first step in explaining carotenoid photochemistries and for understanding the interactions between carotenoids and other molecules in photobiological processes. The primary focus of this chapter is the energetics of carotenoid excited electronic states and their dependence on conjugation length, the presence of substituents, isomeric structure, and solvent environments. Issues regarding excited state dynamics, e.g., the rates of radiative and nonradiative decay, also will be discussed to the extent that they contribute to our understanding of carotenoid spectroscopy. Other chapters describe the importance of carotenoids in photobiological processes and provide further motivation for understanding their excited states.

A great deal of what we know about the low energy excited states of the carotenoids involved in photobiology can be traced to the high-resolution optical spectroscopy of model polyenes. The organization of this chapter parallels the recent evolution of the field and, in large part, follows the

Abbreviations: (0-0) – transition between zero-point vibrational levels of two electronic states (= electronic origin); HOMO – highest occupied molecular orbital; LUMO – lowest unoccupied molecular orbital; PPP-CI – PPP-MO Theory which includes configuration interaction; PPP-MO – Pariser Parr Pople Molecular Orbital Theory; S_0 , S_1 , S_2 – ground (S_0) and excited (S_1 , S_2) electronic singlet states; T_1 , T_2 – excited electronic triplet states; λ_{max} – wavelength of maximum absorption or maximum emission intensity for an electronic transition (in general, $\lambda_{max} \neq \lambda_{(0-0)}$) λ_{max} refers to a vertical transition, i.e., an electronic transition between states with the same geometry.

progression toward structural complexity illustrated by Fig. 1. Incremental increases in conjugation lengths and the addition of substituents provide a relatively continuous transition from the electronic states of octatetraene to molecules such as spheroidene and β -carotene. It comes as no surprise that the level of precision in understanding and describing polyene excited states decreases with increasing molecular complexity. Nevertheless, the experimental data on the longer carotenoids now is of sufficient quality to allow careful answers to questions regarding the energies of carotenoid singlet and triplet states and the ability of these states to serve as energy donors or acceptors in photosynthetic systems.

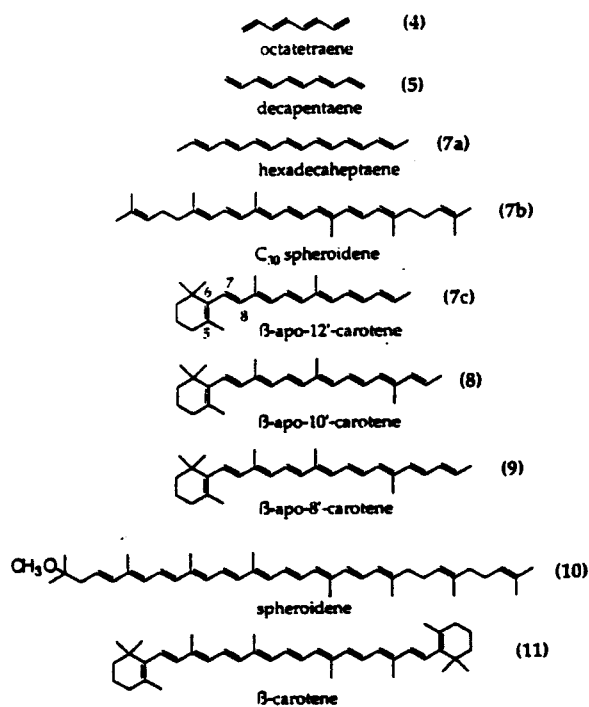


Fig. 1. Representative polyenes and carotenoids. Boldfaced numbers refer to the number of conjugated double bonds.

II. Low-Energy, Excited Singlet States in Polyenes

A. Theoretical Descriptions of Polyene Singlet States

Theoretical descriptions of polyene/carotenoid excited electronic states have been well summarized in several recent reviews (Hudson et al., 1982; Orlandi et al., 1991; Kohler, 1993a,b; Frank and Cogdell, 1996; Koyama et al., 1996). Most, if not all, of our current understanding of the photochemistry of carotenoid singlet states can be described in terms of the simple energy level scheme given in Fig. 2. Transitions to higher energy singlet states (S_3 , S_4 , etc.) also are observed in carotenoid spectroscopy, e.g., the intensity of 'cis-peaks' provides a useful diagnostic tool in identifying isomers (Zechmeister, 1962). However, the photochemistry of carotenoids is dominated by processes originating from low-lying energy levels, and this chapter will restrict its attention to the singlet states depicted in Fig. 2. Symmetry labels for the electronic states are based on the C_{2h} geometry of simple *all-trans*-polyenes. These designations and their implications for transition intensities, radiative lifetimes, etc. also work remarkably well for a wide range of unsymmetric, *cis* and *trans* polyenes and carotenoids, and the A_g and B_u labels will be used throughout this review.

The $S_2(1^1B_u)$ states are well described by simple

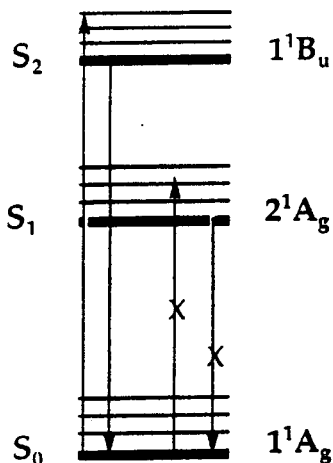


Fig. 2. The ordering of low-energy singlet states in polyenes and carotenoids. Labels refer to molecules belonging to the C_{2h} point group. Arrows with x's refer to symmetry-forbidden electronic transitions.

molecular orbital treatments as being of HOMO \rightarrow LUMO parentage, and the symmetry-allowed, $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transitions have long been appreciated to be responsible for the colors of long polyenes and carotenoids. Indeed, the realization that the $S_0 \rightarrow S_2$, infinite-polyene limit of ~ 600 – 700 nm (Kohler, 1993b; Kohler and Samuel, 1995) is related to the alternation of carbon-carbon bond lengths was one of the early accomplishments of the application of simple Hückel and free-electron models to linearly-conjugated, π -electron systems (Kuhn, 1948; Labhart, 1957; Murrell, 1963). However, satisfactory descriptions of the $S_1(2^1A_g)$ states demand a detailed consideration of the correlation of electron-electron interactions. Within molecular orbital theory, even a qualitative explanation of why $E(2^1A_g) < E(1^1B_u)$ requires the interaction of both singly (e.g., HOMO $- 1 \rightarrow$ LUMO and HOMO \rightarrow LUMO $+ 1$) and doubly (e.g., HOMO, HOMO \rightarrow LUMO, LUMO) excited configurations (D-CI). Quantitative agreement between theoretical and experimental 2^1A_g energies requires even more extensive configuration interaction. For example, the inclusion of all single through quadruply excited configurations (Q-CI) explains the slight increase in $(E(1^1B_u) - E(2^1A_g))$ as a function of increasing polyene length (Tavan and Schulten, 1979). However, the computational effort for calculations at this level of CI increases exponentially with the number of π -electrons, and high level CI calculations have proven prohibitive for polyenes with more than five or six double bonds.

For longer polyenes, multireference double excitation configuration interaction (MRD-CI) approximations have been employed which select the most important electronic configurations and 'balance' the effects of electron correlation in the ground and excited states. The MRD-CI methods provide results comparable to Q-CI or full -CI calculations on short polyenes (Zoos and Ramasesha, 1984) and have allowed calculations to be extended to molecules with up to eight double bonds (Tavan and Schulten, 1986). These calculations also predict a small increase in the $(E(1^1B_u) - E(2^1A_g))$ energy gap with increasing conjugation. The calculated energy gaps (~ 6300 cm^{-1} for octatetraene; ~ 7100 cm^{-1} for hexadecaoctaene) are in reasonable agreement with gas phase experimental values (6604 cm^{-1} for octatetraene (Petek et al., 1994); ~ 9000 cm^{-1} for hexadecaoctaene (Snyder et al., 1985)). However, quantitative comparisons between calculations and spectra are not straightforward: 1.) Calculations of electronic

energy differences refer to 'vertical transitions' in which excited states retain ground state geometries, whereas the experimentally observed energies refer to transitions between the zero-point vibrational levels ((0-0) bands). Vertical transition energies can be converted to (0-0) energies in specific cases, though the corrections depend both on the transition involved and the length of conjugation. 2.) Theoretical calculations presumably refer to isolated molecules, while the experimental data available for longer polyenes generally are limited to spectra obtained in solutions. Solvation causes a significant reduction in the ($E(1^1B_u) - E(2^1A_g)$) energy difference due to the preferential stabilization of the 1^1B_u state. For example, ΔE decreases from 6604 cm^{-1} (Petek et al., 1994) to 3540 cm^{-1} when gaseous *all-trans*-octatetraene (4) is placed into a substitutional site in a crystal of 4.2 K *n*-octane (Granville et al., 1980). (Note that *all-trans*-octatetraene is the only polyene for which the $S_0(1^1A_g) \rightarrow S_1(1^1A_g)$ and $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ electronic origins have been directly observed in both the gas and condensed phases. In fact, a great deal of what we know about the low energy, excited electronic states of polyenes and carotenoids is based on the highly detailed spectroscopic studies of this prototype.) For longer polyenes and carotenoids, for which gas phase spectral measurements are not feasible, it is possible to estimate corrections to transition energies for solvent effects (see below) to allow direct comparison with theoretical calculations. One additional consideration is that the parameters used in the PPP-MRD-CI and other semi-empirical methods may in part be based on spectra of molecules in condensed phases. 3.) Comparison of theory with carotenoid electronic energy differences is further complicated by the differential effects of substitution (e.g., methyl groups and β -ionylidene rings) on the S_0 , S_1 , and S_2 states. Some attempts have been made to empirically account for the effects of substitution (Kohler, 1993a,b). However, this is complicated by limitations in the accuracy of data available for the zero-point 2^1A_g energies of longer polyenes and problems in correcting 1^1B_u and 2^1A_g energies for comparisons in common solvents and temperatures.

In summary, theory gives at least a semi-quantitative explanation for why Fig. 2 applies to a wide range of polyene systems and provides a useful guide for understanding relative energies, the symmetry-forbidden nature of the $S_0 \leftrightarrow S_1$ transitions, and the re-arrangement of π -bond orders in carotenoid

excited states (Schulten et al., 1976). Nevertheless, the further development of an accurate catalog of 2^1A_g energies will continue to rely on their spectroscopic detection, especially for carotenoids employed in photobiological processes. Identification of (0-0) energies is particularly important, since the zero-point vibrational states are the starting points for photochemistry and energy transfer in thermally equilibrated systems.

B. The Experimental Observation of Polyene Singlet States

1. One-Photon Spectroscopy/Vibronic Structure

The connection between experiment and the energy level diagram summarized in Fig. 1 is illustrated by the low temperature absorption and fluorescence spectra of *all-trans*-hexadecaheptaene (Fig. 3). The vibronic structure exhibited in these spectra generally is broadened in spectra of carotenoids, particularly for molecules such as β -carotene where non-planarities between the central polyene chain and terminal cyclohexenyliene rings result in a distribution of absorbing and emitting species (Christensen and Kohler, 1973; Hemley and Kohler, 1977). The well-resolved spectra of unsubstituted, model polyenes facilitates the unambiguous identification of electronic origins ((0-0) bands) and the precise measurement of 1^1B_u and 2^1A_g electronic energies. Most characteristic of spectra of short, model polyenes is the gap between the onsets ((0-0)'s) of the strongly allowed, $1^1A_g \rightarrow 1^1B_u$ absorption (molar absorptivities of $\sim 10^5 \text{ L mole}^{-1} \text{ cm}^{-1}$) and the $2^1A_g \rightarrow 1^1A_g$ emission. Although the fluorescence quantum yield for hexadecaheptaene has not been measured, the $S_1 \rightarrow S_0$ yields show a steady fall-off with increasing conjugation, ranging from ~ 1 in octatetraene (Gavin et al., 1978) to $< 10^{-5}$ in molecules such as β -carotene (Bondarev and Knyuksho, 1994; Andersson et al., 1995).

The vibronic features of these spectra are worth noting. (Higher-resolution versions of the hexadecaheptaene spectra, obtained in low-temperature, mixed crystals, have been discussed by Simpson, et al. (1987)). The absorption and emission spectra presented in Fig. 3 are dominated by combinations of totally symmetric (a_g) C-C and C=C stretching modes with vibrational frequencies of ~ 1200 and $\sim 1600 \text{ cm}^{-1}$. These details are more easily identified in the $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ fluorescence spectrum which is

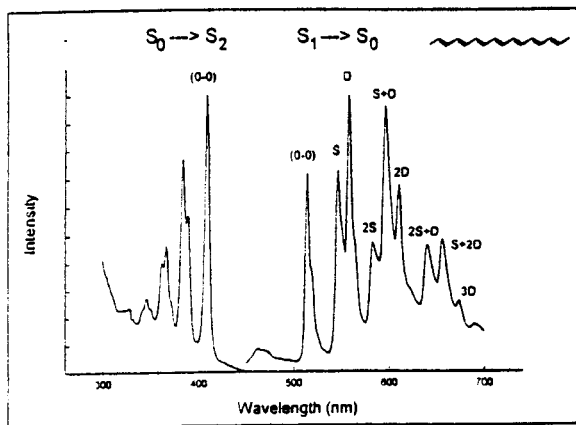


Fig. 3. Fluorescence and fluorescence excitation spectra of *all-trans*-hexadecaheptaene (7a) in 77 K *n*-pentadecane. The fluorescence excitation spectrum was obtained by exciting at 414 nm, and the fluorescence excitation spectrum monitored the emission intensity at 558 nm. Vibronic structure in the fluorescence spectrum has been assigned to combinations of carbon-carbon single (S) and double (D) bond stretching vibrations.

better-resolved and shows Franck-Condon maxima that characteristically involve at least one quantum of the double bond stretch. In broader spectra the vibronic features corresponding to single and double bond stretches often coalesce into progressions in what appear to be a single, intermediate frequency of 1300–1400 cm^{-1} , depending on the length of conjugation and the transition involved. Note that the absorption spectrum of the heptaene has its maximum intensity in the (0-0) band, most likely reflecting the smaller geometry change experienced in the $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transition. This is consistent with theory which predicts a more significant transposition of π -bond orders in the 2^1A_g state (Schulten et al., 1976). It also is important to note that the vibronic features in Fig. 3, those seen in low-temperature spectra of mixed crystals (Simpson et al., 1987), and the highly detailed vibronic development observed in high-resolution spectra of isolated tetraenes in supersonic expansions (Petek et al., 1995) all are consistent with planar 2^1A_g and 1^1B_u excited states in longer polyenes. There is no evidence in long polyenes and carotenoids for the substantial deviations from planarity experienced by the excited states of dienes and trienes (Dormans et al., 1987; Zerbetto and Zgierski, 1990; Orlandi et al., 1991).

The characteristic Franck-Condon envelopes and vibronic signatures of polyene transitions observed in the fluorescence spectra of molecules such as

hexadecaheptaene prove to be critical in interpreting the weaker, more poorly resolved emissions of longer carotenoids. It also is important to understand why the (0-0) band is so prominent (Fig. 3) in this supposedly forbidden $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ transition. First, it should be noted that other than in high resolution experiments (e.g., Simpson et al., 1987; Kohler et al., 1988), the $S_0(1^1A_g) \rightarrow S_1(2^1A_g)$ transitions generally are too weak (with transition dipoles and integrated extinction coefficients $\sim 10^{-2}$ – 10^{-3} those of allowed polyene transitions) to be detected in standard absorption or fluorescence excitation measurements. The weakness of these bands confirms that they truly are due to symmetry-forbidden, $g \rightarrow g$ transitions. On the other hand, the selection rules also imply that the (0-0) band should be weak (or nonexistent) with the major part of the spectral intensity built on nontotally symmetric (b_u) 'promoting' modes that mix the 2^1A_g and 1^1B_u electronic states (Geldof et al., 1971).

High-resolution experiments on model polyenes explain the apparent contradiction of observing relatively strong (0-0) bands in symmetry-forbidden electronic transitions. Under isolated-molecule conditions (e.g., supersonic jets) or in the small number of cases where polyenes have been incorporated into crystals that rigorously retain their inversion centers, the $S_0 \leftrightarrow S_1$ (0-0)'s are indeed missing with transitions being built on b_u false origins (Petek et al., 1995). However, the most prominent b_u promoting vibrations are in-plane bending modes of very low frequency, $< 100 \text{ cm}^{-1}$ in long polyenes. Therefore, even for polyenes or carotenoids that retain idealized C_{2h} symmetries, the distinctions between (0-0) bands and b_u false origins will not be detectable, even in relatively well-resolved $S_1 \rightarrow S_0$ spectra such as presented in Fig. 3. In addition, small distortions from C_{2h} symmetry, either due to asymmetric substitutions or solvent perturbations, tend to give the (0-0) bands intensities that are comparable to those of false origins (Christensen and Kohler, 1976; Petek et al., 1995). Furthermore, these bands undergo inhomogeneous solvent shifts due to the distribution of local solvent environments. As a result, the electronic origins of $S_0 \leftrightarrow S_1$ spectra of polyenes in solutions and glasses should be viewed as due to a complicated, unresolved collection of distorted and undistorted molecules all of which contribute to the '(0-0)' bandshapes in typical spectra.

2. Two-Photon Spectroscopy

Two-photon spectra of *all-trans* octatetraene, both in *n*-octane (which provides centrosymmetric mixed-crystal sites) and for isolated molecules in supersonic jets, furnish the most direct proof that the lowest energy transition must be assigned to a $g \rightarrow g$ transition (Granville et al., 1979; Petek et al., 1995). The $S_0 \rightarrow S_1$ transitions are allowed under two-photon excitation conditions, giving relatively intense (0-0) bands and spectra dominated by totally symmetric (a_g) vibrations. Furthermore, in two-photon experiments $g \rightarrow u$ transitions are forbidden, thus enhancing the ability of this technique to locate 2^1A_g states in molecules whose π -electron densities maintain at least an approximate center of inversion. This technique found early success in locating low-energy 2^1A_g states in retinol, retinal, and a retinal Schiff base (Birge et al., 1978). However, there has been remarkably little additional exploitation of two-photon methods to locate 2^1A_g states in other polyenes or carotenoids. This is due, in part, to the weak $S_1 \rightarrow S_0$ emissions from longer polyenes and carotenoids which preclude the detection of their two-photon $S_0(1^1A_g) \rightarrow S_1(2^1A_g)$ absorptions using fluorescence excitation techniques. In addition, the low energies of $S_0(1^1A_g) \rightarrow S_1(2^1A_g)$ transitions in carotenoids requires two-photon experiments to be conducted in the near infrared where one-photon-allowed overtones of solvent vibrations can cause significant interferences (R. R. Birge, personal communication).

3. Solvent Effects

The $S_0(1^1A_g) \rightarrow S_1(2^1A_g)$ and $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transitions undergo shifts to lower energy when gaseous molecules are solvated. Understanding these shifts is important in relating theoretical calculations to transition energies obtained from typical solution spectra. Solvent effects also provide a useful diagnostic tool for distinguishing between allowed and forbidden transitions. The analysis of solvent effects in polyene spectroscopy has been well described by Hudson et al. (1982). For nonpolar polyenes in nonpolar solvents the transition energies should exhibit a linear dependence on solvent polarizability:

$$\bar{\nu}(\text{solvent}) = \bar{\nu}(\text{gas}) - k(n^2 - 1)/(n^2 + 2) \quad \text{Eq. (1)}$$

where $\bar{\nu}$ refers to the transition energy (typically the (0-0) band expressed in cm^{-1}), n is the refractive index of the solvent, and k is constant for a given polyene transition. As discussed by Hudson et al. (1982),

$$k \propto a^{-3}(M + E(\alpha_c - \alpha_g)) \quad \text{Eq. (2)}$$

where a is an effective solute cavity radius, M_{ge} is the electric dipole transition moment for the transition, E is the average transition energy, and $(\alpha_c - \alpha_g)$ is the increase in the polarizability of the polyene upon excitation.

These equations have been tested for several short polyenes and for β -carotene (Sklar et al., 1977; Snyder et al., 1985; Andersson et al., 1991). For $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transitions, k 's are typically 10^4 cm^{-1} . In addition to the linear dependence of transition energies on solvent polarizability, another critical test of Eq. (1) is its ability to predict gas phase ($n=1$) transition energies. For the $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transitions of diphenylpolyenes with one to four double bonds, extrapolations of solution data and transition energies obtained from gas phase measurements agree within experimental error (Hudson et al., 1976, 1982). Equation 1 also accounts for the shifts in spectra in the few cases where solvent studies have been carried out on vibronically-resolved $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ transitions. The k 's are considerably smaller but not insignificant, ~ 1000 – 2000 cm^{-1} for unsubstituted polyenes (Snyder et al., 1985). In the one case (*all-trans*-octatetraene) where the gas phase $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ electronic origin is accurately known ($28,949 \text{ cm}^{-1}$, Petek et al., 1995), the solution phase data provide an excellent extrapolation ($28,970 \pm 100 \text{ cm}^{-1}$) to $n=1$ (Snyder et al., 1985). The data available for short polyenes and diphenyl polyenes in nonpolar solvents thus validate the use of the solvent model described by in Eqs. (1 and 2). This solvent model is particularly important for understanding how the S_1 and S_2 energies are modified when carotenoids are placed in biological matrices, e.g., the protein environments of reaction centers and light-harvesting antenna.

For carotenoids, Andersson et al. (1991) have suggested that the $E(\alpha_c - \alpha_g)$ term dominates the transition dipole term in determining k for the $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ absorption. Although there is considerably less solvent effect information available for $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ transitions (See Snyder et al. (1985) for a

summary.), their relatively large k 's and relatively small transition moments imply that polarizability changes also govern the solvent dependence of these transitions, at least for shorter polyenes. It is somewhat surprising that the k 's do not exhibit a systematic dependence on polyene length (Sklar et al., 1977; Hudson et al., 1982; Snyder et al., 1985). The transition dipole moment and the change in polarizability for the $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transition both are expected to increase with increasing conjugation (Kohler, 1990; Anderson et al., 1991). However, the k 's (at least for the $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transitions) are essentially independent of conjugation length, cf. $k = 11,700 \text{ cm}^{-1}$ for β -carotene (Andersson et al., 1991) and $k = 10,900 \text{ cm}^{-1}$ for octatetraene (Snyder et al., 1985). A plausible explanation is that the increases in transition moments and polarizability upon optical excitation are counteracted by comparable increases in molecular volumes ($\propto \alpha^3$) that also should scale with the length of conjugation.

III. Low-Energy, Excited Singlet States in Carotenoids

Extending the spectroscopic investigations of polyene singlet states to the carotenoids employed in photobiology is hampered by several 'deficiencies' of longer, more highly substituted conjugated systems. Foremost is the marked decrease in fluorescence quantum yields with increasing conjugation. The dominance of non-radiative decay processes makes it increasingly difficult to detect the symmetry-forbidden, $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ transitions, let alone to confirm their identity using standard fluorescence excitation techniques. Furthermore, increasing the conjugation length leads to a predominance of higher energy, $S_2(1^1B_u) \rightarrow S_0(1^1A_g)$ emissions with low-energy, vibronic tails that mask the $S_1 \rightarrow S_0$ fluorescence. In addition to issues of fluorescence intensity, the electronic spectra of naturally occurring carotenoids tend to be significantly broadened, making it even more difficult to observe and identify the weak fluorescence signals from these systems. These problems often are exacerbated by interferences from the relatively strong emissions of shorter polyene breakdown products and other impurities.

A. The Electronic Spectroscopy of Short Carotenoids

Some of the differences between the electronic spectroscopy of simple polyenes and that of carotenoids are illustrated in Fig. 4 which compares the low-temperature absorption and fluorescence spectra of three heptaenes: hexadecaheptaene (7a), a synthetic C_{30} spheroidene (7b), and an apo-carotene (7c). Spectra of hexadecaheptaene are well resolved with well-defined electronic origins, allowing the accurate measurement of the $S_1(2^1A_g)$ and $S_2(1^1B_u)$ electronic energies. The addition of methyl substituents to form the isoprenoid, C_{30} spheroidene (7b) results in broadening of the vibronic bands and systematic shifts of transitions to lower energy. The $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transition is considerably more sensitive to methyl substitution than the $S_0(1^1A_g) \rightarrow S_1(2^1A_g)$ transition. This is a common feature of polyene/carotenoid spectroscopy, mirroring the relative sensitivities of the 2^1A_g and 1^1B_u energies to solvent perturbations. Introduction of the terminal β -ionylidene ring in the apo-carotene further broadens the spectra and shifts the $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transition to substantially higher energy relative to the absorption spectrum of the corresponding C_{30} spheroidene. The apo-carotene $S_1(1^1A_g) \rightarrow S_0(2^1A_g)$ bands experience a significantly smaller blue shift relative to the spheroidene.

The loss of resolution in the apo-carotene spectra can be traced to repulsions between methyl groups on the β -ionylidene ring and the hydrogen atom at the end of the $C_{7,8}$ double bond (Fig. 1) in the polyene chain (Christensen and Kohler, 1973; Hemley and Kohler, 1977). These interactions twist the $C_{6,7}$ single bond, forcing the double bond in the ring ($C_{5,6}$) out of the plane formed by the other carbon-carbon double bonds. X-ray structures of carotenoid single crystals provide a direct measure of the significant (and variable) nonplanarities between rings and side chains in retinoic acid and retinal (Stam and MacGillavry, 1963; Gilardi, et al., 1971). The potential energy along the 5-6-7-8 dihedral angle apparently is both shallow and flat, resulting in a distribution of conformations in solutions and glasses. As a result, solutions of apo-carotenes, β -carotene, etc. typically contain a distribution of conformers with a range of effective conjugation lengths and transition energies. This simple model not only accounts for the relative broadness of carotenoid optical spectra but also

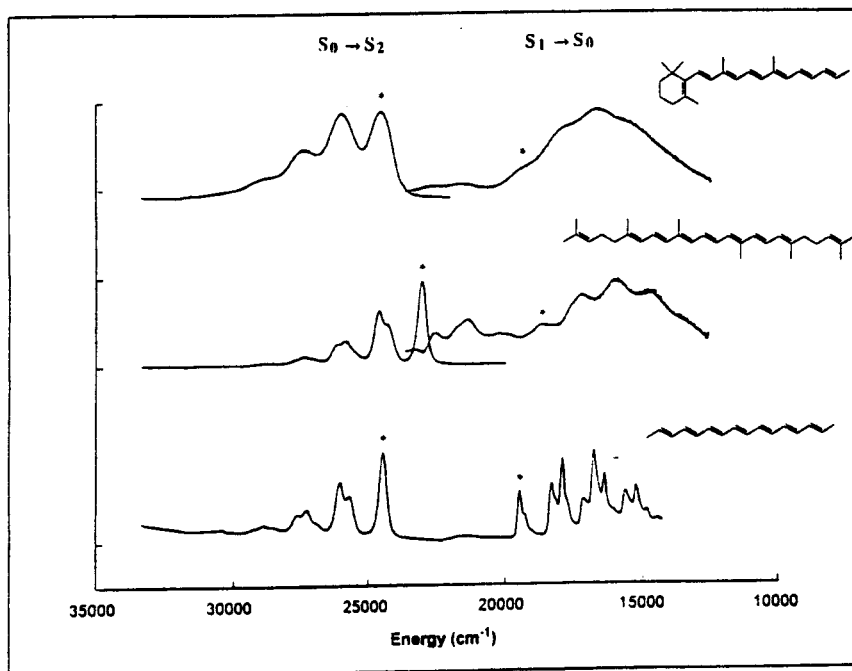


Fig. 4. Comparison of absorption and fluorescence spectra of hexadecaheptaene (7a), a C_{30} spheroidene (7b), and β -apo-12'-carotene (7c). Spectra were obtained at 77 K in an EPA glass (ether/isopentane/ethanol, 5/5/2, v/v/v). Electronic origins are designated by asterisks.

explains the significant blue shift in the apo-carotene (7a) relative to the spheroidene (7b). The C_{30} spheroidene enjoys the full effect of seven conjugated double bonds, while the apo-carotene spectrum is due to a distribution of conjugation lengths which average between six (90° rotation about $C_{6,7}$) and seven (no rotation) conjugated bonds. Empirical estimates of the average loss in effective conjugation due to the presence of a β -ionylidene ring range from 0.15 (Kohler, 1993) to 0.8 (Hirayama, 1955). These effects also explain the small red shift in the $S_0 \rightarrow S_2$ absorption spectrum of lycopene relative to that of β -carotene and our perception of differences in the colors of these pigments in plants. In addition, this model accounts for the systematic increase in the resolution of carotenoid $S_0 \rightarrow S_2$ spectra with increasing conjugation length. The leveling-off of $S_0 \rightarrow S_2$ transition energies for large N implies that nonplanarities of the terminal double bonds have a decreasing impact on the spread in transition energies (Hemley and Kohler, 1977).

Comparison of the spectra of hexadecaheptaene (7a) and the apo-heptaene (7c) show fortuitous agreements in $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ and $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ transition energies. For heptaenes the red shift induced by methyl substituents is effectively

counterbalanced by the blue shift due to nonplanarities and the loss in effective conjugation length. This suggests that model polyenes may provide reasonable models for estimating the excited state energies of apo-carotenes (including molecules such as β -carotene) with the same formal number of double bonds, though more work is needed to better understand the effects of substituents on the 2^1A_g and 1^1B_u energies of longer carotenoids.

B. The Electronic Spectroscopy of Long Carotenoids

1. Cross-Over from $S_1 \rightarrow S_0$ to $S_2 \rightarrow S_0$ Emissions in Carotenoids and Polyenes of Intermediate Length

The major differences between the optical spectroscopy of model polyenes and short apo-carotenes and the longer, more conjugated carotenoids employed in photobiology are illustrated in Fig. 5. Most striking is the cross-over from the $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ emission observed for the apo-heptaene (7c) to the dominant, $S_2(1^1B_u) \rightarrow S_0(1^1A_g)$ fluorescence of the analogous apo-nonaene (9). Similar changes from $S_1 \rightarrow S_0$ to $S_2 \rightarrow S_0$ emissions have been noted in

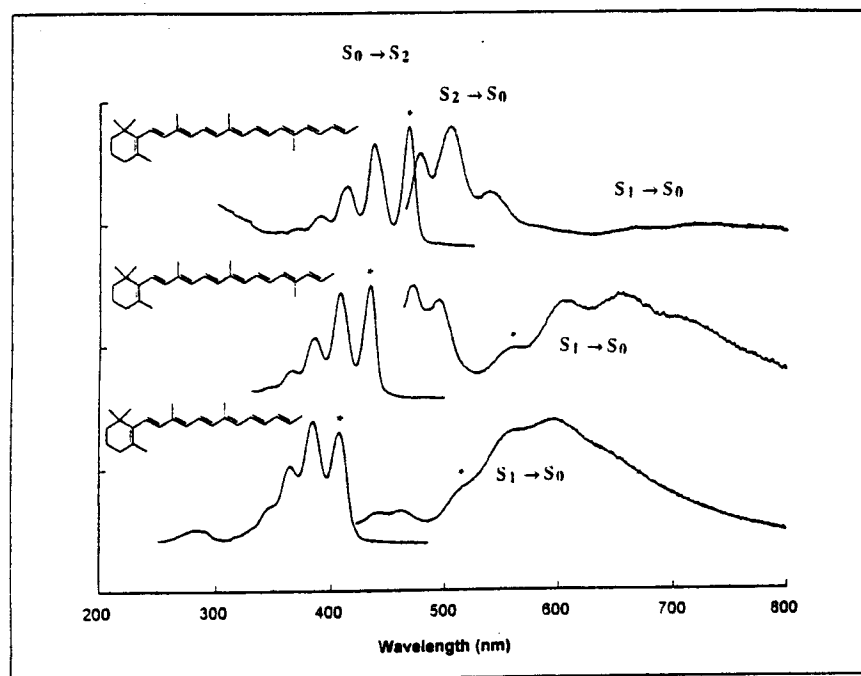


Fig. 5. Comparison of absorption and fluorescence spectra of β -apo-12'-carotene (7c), β -apo-10'-carotene (8), and β -apo-8'-carotene (9). Spectra were obtained at 77 K in an EPA glass. Electronic origins are designated by asterisks.

simple polyenes (Snyder et al., 1985), apo-carotenols (Cosgrove et al., 1990), spheroidenes (DeCoster et al., 1992), and analogs of β -carotene (Anderson et al., 1995). Fluorescence quantum yields are sensitive to structural details, though the relatively abrupt change to $S_2 \rightarrow S_0$ emissions invariably occurs for molecules with seven or eight conjugated bonds. This has the important practical consequence for photobiology that the 2^1A_g states of molecules such as spheroidene (10) and β -carotene (11) are difficult to detect using routine fluorescence techniques.

The characteristic change from $S_1 \rightarrow S_0$ to $S_2 \rightarrow S_0$ emissions in longer polyenes originally was attributed (Snyder et al., 1985; Cosgrove et al., 1990) to the increase in the S_2 - S_1 energy difference with increasing conjugation length. This presumably results in a decrease in the rate of $S_2 \rightarrow S_1$ internal conversion following the well-known 'energy-gap law' (Englman and Jortner, 1970). For large energy gaps internal conversion rates become sufficiently small to allow the strongly allowed $S_2 \rightarrow S_0$ fluorescence to compete with radiationless decay processes, leading to violations of Kasha's Rule (Turro, 1978; Wayne, 1991). (Kasha's Rule states that for large molecules in condensed phases, radiationless processes from higher energy excited states ($S_2, S_3, \dots; T_2, T_3, \dots$) are

so rapid that all radiative and photochemical processes originate from the lowest energy electronic state of a given multiplicity, i.e., S_1 or T_1 .) The energy gap model accounts for $S_2 \rightarrow S_0$ emissions in several aromatic molecules all of which are characterized by large S_2 - S_1 energy differences (Englman and Jortner, 1970).

Long polyenes and carotenoids, at first glance, are likely violators of Kasha's Rule. The S_2 - S_1 energy difference at which $S_2 \rightarrow S_0$ fluorescence is observed ($> 5000 \text{ cm}^{-1}$) is consistent with the thresholds for S_2 emissions in a variety of organic and inorganic systems. However, the rather abrupt changeover from $S_1 \rightarrow S_0$ to $S_2 \rightarrow S_0$ emissions is not easily reconciled with the very modest changes in S_2 - S_1 energy differences in previously studied polyene/carotenoid series (Snyder et al., 1985; Cosgrove et al., 1990; DeCoster et al., 1992; Anderson et al., 1995). Indeed, the three apo-carotenoids whose spectra are presented in Fig. 5 have S_2 - S_1 energy gaps that are almost identical ($5100 \pm 150 \text{ cm}^{-1}$, R. Christensen, unpublished). Given a constant S_2 - S_1 energy gap, the rate of $S_2 \rightarrow S_1$ radiationless decay should be relatively insensitive to conjugation length. In fact, the expected increase in the density of vibrational 'accepting' modes in longer apo-carotenoids (generally thought to

be C-C stretches, Orlandi et al., 1991) argues that $S_2 \rightarrow S_1$ internal conversion rates should increase in longer apo-carotenes. Recent investigations of $S_2 \rightarrow S_0$ fluorescence quantum yields in carotenes (Andersson et al., 1995) and spheroidenes (Frank et al., 1997) indicate $S_2 \rightarrow S_1$ internal conversion rates that are remarkably insensitive to conjugation length over the range $N=5$ to $N=13$. The 'cross-over' to $S_2 \rightarrow S_0$ fluorescence in these systems and in the longer apo-carotenes (Fig. 5) thus is most easily explained by increases in the rates of $S_1 \rightarrow S_0$ nonradiative decay due to a combination of smaller S_1-S_0 energy gaps and the increased density of S_0 accepting modes in larger molecules. This leads to the disappearance of $S_1 \rightarrow S_0$ fluorescence, allowing the weak, residual $S_2 \rightarrow S_0$ fluorescence to dominate the emissions of longer carotenoids. Further insights on $S_2 \rightarrow S_1$ internal conversion rates in spheroidenes and carotenes are provided by Andersson et al. (1995) and Frank et al. (1997).

2. Extrapolation of $S_2 \leftrightarrow S_0$ and $S_1 \leftrightarrow S_0$ Transition Energies to Long Carotenoids: The Infinite Polyene Limit

The absence of detectable $S_1 \rightarrow S_0$ emissions from long carotenoids thwarts the direct detection of the 2^1A_g states in molecules such as violaxanthin ($N=9$), antheraxanthin ($N=10$), spheroidene ($N=10$), zeaxanthin ($N=11$), β -carotene ($N=11$), spirilloxanthin ($N=13$), etc. Since an accurate knowledge of 2^1A_g energies is essential for understanding the roles these molecules play in photosynthetic systems, it has been tempting to extrapolate from the 2^1A_g energies of shorter, more fluorescent systems. Fig. 6 shows the (0-0) energies as a function of the number of double bonds for methyl-substituted polyenes and spheroidenes (DeCoster et al., 1992; Fujii et al., 1998). Simple extrapolations of these kinds of plots prove useful in analyzing the weak $S_1 \rightarrow S_0$ emissions from longer, more conjugated systems, particularly in estimating the positions of (0-0) bands in weak and/or poorly resolved spectra. For example, simple linear extrapolations of S_2-S_1 energy gaps combined with measurements of $S_0 \rightarrow S_2$ (0-0)'s or the more elaborate extrapolation schemes described below should provide reliable estimates of the S_1 energies of carotenoids employed in photosynthetic systems, i.e., for $9 \leq N \leq 13$.

More systematic extrapolation approaches have relied on theoretical models which suggest that

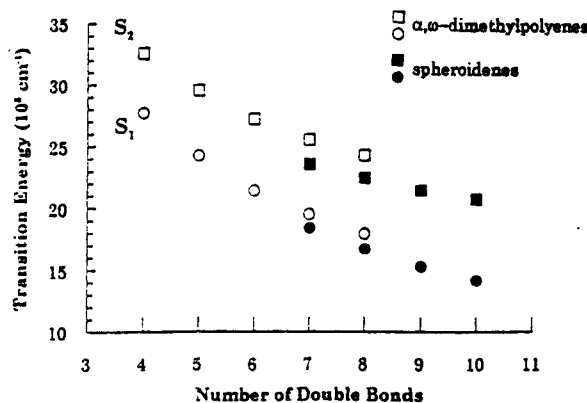


Fig. 6. $S_1 \rightarrow S_0$ ($1^1A_g \rightarrow 2^1A_g$) and $S_0 \rightarrow S_2$ ($1^1A_g \rightarrow 1^1B_u$) transition energies for α,ω -dimethylpolyenes and spheroidenes as a function of conjugation length. Transition energies are the electronic origins (0-0 bands) observed for $S_1 \rightarrow S_0$ and $S_0 \rightarrow S_2$ transitions in room temperature methanol. (The S_1 energy of spheroidene ($N=10$) was obtained in *n*-hexane by Fujii et al. (1998).) Dimethylpolyene data are from Morey and Christensen (unpublished) and Kohler et al. (1988). Spheroidene data are from DeCoster et al. (1992) and from Fujii et al. (1998). S_2 energies are known to ± 50 cm^{-1} . S_1 energies have uncertainties of ± 100 cm^{-1} for the dimethylpolyenes, ± 200 cm^{-1} for the spheroidenes.

transition energies should vary as N^{-1} where N is the number of conjugated double bonds. The simplest versions of the free electron and Hückel MO models predict that, in the absence of bond alternation, the one-electron, $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ transition energies should exhibit a simple $(2N+1)^{-1}$ dependence on polyene length with transition energies approaching zero for infinite polyenes (Kuhn, 1948; Murrell, 1963). Bond alternation and the resulting asymptotic approach to non-zero transition energies suggest that the $S_0 \leftrightarrow S_2$ transition energies of long polyenes should be modeled by $E = A + BN^{-1}$ (or $E = A + B(2N+1)^{-1}$). Tavan and Schulten (1987) have argued that similar dispersion relations should apply to $S_1 \leftrightarrow S_0$ transition energies, though extending the form of the $S_0 \leftrightarrow S_2$ extrapolations to transitions involving correlated, $1^1A_g \rightarrow 2^1A_g$ excitations has no a priori justification. Nevertheless, the limited amount of reliable data for the 2^1A_g energies of short polyenes does show a linear dependence on N^{-1} , and these fits have been extended to estimate 2^1A_g energies in longer, nonfluorescent polyenes. An interesting by-product of such fits is their use to predict 2^1A_g energies and $S_2 - S_1$ energy gaps in infinite polyenes, spheroidenes, and carotenes.

A review of recent extrapolations is summarized

in Table 1. The substantial variation in extrapolated ($N \rightarrow \infty$) 2^1A_g and 1^1B_u energies illustrates the perils of such extrapolations, particularly for 2^1A_g states for which there is so little theoretical or experimental guidance on how transition energies should depend on the length of conjugation. Table 1 also reformulates the as yet unresolved issue of the differences between the electronic energies of simple polyenes and carotenoids, in particular the extent to which methyl substituents and terminal, β -ionylidene rings modify the energies for a particular N .

The early work of Hemley and Kohler (1977) used $E = A + B/N$ to fit the $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$, (0-0) transition energies of unsubstituted polyenes with four through ten double bonds in a common hydrocarbon solvent (isooctane). This later proved to be consistent with Kohler's simple theoretical model (1990) which empirically adjusted the configuration interaction matrix elements used to calculate the 2^1A_g and 1^1B_u energies for unsubstituted, α, ω -dialkyl

substituted, and α, ω -diphenyl substituted polyenes. The only molecules considered in the data set were those for which (0-0) transition energies were precisely known from low-temperature, mixed-crystal spectra in *n*-alkane solvents. Using an iterative, least squares procedure that corrected conjugation lengths for the effects of alkyl and phenyl substitution, Kohler reproduced $\sim 25 S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ and $S_0(1^1A_g) \leftrightarrow S_1(2^1A_g)$ (0-0) transition energies. The empirically derived, best-fit parameters then were applied to calculate the infinite polyene transition energies presented in Table 1.

Kohler (1993b) later used simple N^{-1} fits and the concept of an 'effective' conjugation length ($N_{\text{eff}} = N \pm \delta N_{\text{substituent}}$) to develop universal fits of the 2^1A_g and 1^1B_u energies of different polyenes series. The goal of this work was to develop empirical equations with well-defined rules for calculating N_{eff} that would account for (and predict) the 2^1A_g and 1^1B_u energies of any substituted polyene or carotenoid. For example,

Table 1. Extrapolations of carotenoid/linear polyene transition energies to the infinite polyene limit

2^1A_g ($n = \infty$) in cm^{-1}	1^1B_u ($n = \infty$) in cm^{-1}	
	16,500 ^a	unsubstituted polyenes in iso-octane ($E = A + B/N$)
	14,400 ^b	α, ω - <i>tert</i> -butyl polyenes in pentane ($E = A + B/N$)
8,710 ^c	15,878 ^c	least squares fit to Hückel model with empirical adjustment of configuration-interaction matrix elements to account for the effects of α, ω -dialkyl substitution (based on data obtained in low temperature, <i>n</i> -alkane solvents)
6,800 ^d	14,250 ^d	$E = A + B/N_{\text{eff}}$ where N_{eff} attempts to correct for the effects of a wide range of substituents (Solvents and temperatures are not clearly specified.)
6,500 ^e		unsubstituted and methyl-substituted polyenes in polar and nonpolar solvents and over a range of temperatures $E = A + B/(2N+1)$
4,800 ^e		carotenoids and spheroidenes in a variety of solvents and temperatures $E = A + B/(2N+1)$
7,790 ^f	13,630 ^f	carotenes in 3-methyl pentane glasses S_1 energies of long carotenes were extrapolated from the energy gap law. $E = A + B/N$
3,470 ^f	10,980 ^f	$E = A + B/N + C/N^2$
3,802 ^g	10,955 ^g	spheroidenes in methanol and <i>n</i> -alkanes S_1 energies of long spheroidenes were extrapolated from the energy gap law. $E = A + B/(N + C)$
-4,200 ^h	2,400 ^h	diphenylpolyenes in toluene ($N = 2-7$) $E = A + B/(N + C)^D$

^a Hemley and Kohler, 1977; ^b Knoll and Schrock, 1998; ^c Kohler, 1990; ^d Kohler, 1993b; ^e Koyama et al., 1996; ^f Andersson and Gillbro, 1995; ^g Frank et al., 1997; ^h Bachilo et al., 1998

to rationalize the 1^1B_u energies of molecules such as β -carotene, N was reduced to $N_{\text{eff}} = N - 0.3$ to account for the loss of effective conjugation due to the out-of-plane rotations of the terminal β -ionylidene rings. Similar empirical corrections were made for alkyl and phenyl substituents. Although the resulting fits of E vs. N^{-1} are generally of good quality, the discrepancies with the parameters (e.g., $N=\infty$ energies) obtained from data on simple, unsubstituted polyenes (Hemley and Kohler, 1977; Kohler, 1990) indicates some systematic shortcomings in accounting for substituents in this manner. One of the problems with this approach is illustrated in the difference between using $N_{\text{eff}} = N - 0.3$ for the 1^1B_u energies and $N_{\text{eff}} = N + 0.5$ for the 2^1A_g energies to correct for the presence of double bonds rings in molecules such as β -carotene. It is plausible that effective conjugation lengths may not be the same for every electronic transition, but an increase in conjugation length in 2^1A_g due to nonplanarity has no physical basis nor any support from spectra such as presented in Fig. 4. In spite of these obvious shortcomings, further development of universal 1^1B_u and 2^1A_g energy curves has considerable appeal. Refinement of this approach will require that energies be related to a common solvent and temperature and clearly awaits more reliable spectroscopic data on 2^1A_g (0-0) transition energies in longer polyenes and carotenoids.

Recent attempts at extrapolating carotenoid energies require careful examination. Andersson and Gillbro (1995) combined estimates of (0-0) energies obtained from the fluorescence spectra of short, mini-carotenes (including β -carotene) with estimates of 2^1A_g energies of homologous, longer members of this series, obtained from lifetime measurements and empirical extrapolations of the energy-gap law (see below). The 2^1A_g and 1^1B_u energies then were fit to both two and three term expansions in $1/N$ to obtain estimates of the long polyene limits for the two states. The two-term ($E = A + B/N$) expansions give parameters that are comparable with those of Kohler. However, it is noteworthy that the two-term extrapolation of Andersson and Gillbro leads to a $1^1B_u - 2^1A_g$ energy gap that is essentially independent of carotenoid length. In order to improve the fits to the (0-0)'s for the longer members of the series (whose energies were estimated from extrapolations based on the energy gap law - see below), a three term expansion was used which significantly lowered the

2^1A_g and 1^1B_u energies in the long polyene limit. It is important to stress that there is no theoretical justification for a three term expansion and that the inclusion of the additional term largely appears to have been dictated by the only two carotenoids whose 2^1A_g energies could not be determined directly.

Frank et al. (1997) obtained comparable data for a homologous series of spheroidenes with seven to thirteen double bonds. It is significant that the $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ (0-0) band can be directly observed only for the shortest three of the seven molecules in this series with the remaining (0-0) energies being estimated from energy-gap law extrapolations. These energies as well as the $S_0(1^1A_g) \rightarrow S_2(1^1B_u)$ (0-0)'s then were fit to $A+B/(N+C)$ with the results indicated in Table 1. The large ($\sim 5000 \text{ cm}^{-1}$) differences between the long chain ($N = \infty$) 2^1A_g and 1^1B_u energies of simple polyenes (Kohler, 1990, 1993b) and the energies obtained by Andersson and Gillbro and by Frank et al. have been attributed to the stabilizing effects of the methyl substituents in the carotenoid systems. However, there is little support for substituent effects of this magnitude (e.g., $C = 2$ for the $S_0 \rightarrow S_2$ transitions) in the spectra of shorter model systems (Fig. 4).

At this point, most of the discrepancies in the long-polyene/long-carotenoid extrapolations summarized in Table 1 should be attributed to the differences in the fitting functions rather than fundamental differences in the data. It is important to reiterate that there is no theoretical basis for either of the three parameter extrapolations employed for the carotene or spheroidene data. The recent use of four parameter functions to fit the S_2 and S_1 energies of diphenylpolyenes (Bachilo et al., 1998) further illustrates the limitations of arbitrary, phenomenological functions to describe polyene/carotenoid electronic energies. In this later case, the large N extrapolations lead to negative S_1 energies, providing a strong reminder of the lack of a physical basis for such fits. At a minimum, it will be important to subject simple polyenes and carotenoids to the same analysis to explore the systematic effects of substitution and conjugation length on the electronic energies of these systems. Careful use of extrapolation procedures also must consider the relative weights of data points. There are substantial differences in the uncertainties of S_1 energies obtained from well-resolved spectra and those estimated from energy gap extrapolations. These uncertainties should be

reflected in the statistical weights used in least squares fits to properly evaluate how electronic energies depend on the length of conjugation.

3. Use of Lifetime Measurements and the Energy-Gap Law to Estimate 2^1A_g Energies in Long Carotenoids.

The reduction of $S_1 - S_0$ energy differences and the increased density of S_0 vibrational accepting modes work together to enhance the rates of $S_1 \rightarrow S_0$ radiationless decay in longer polyenes and carotenoids. As described above, this accounts for the steady fall-off in $S_1 \rightarrow S_0$ fluorescence yields in going from octatetraene ($\phi_f \sim 1$ in low temperature glasses (Gavin et al., 1978)) to molecules such as β -carotene ($\phi_f < 10^{-5}$, Andersson et al., 1995). The extremely weak $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ emissions in polyene/carotenoids with $N > 9$, i.e., in almost all carotenoids of photobiological interest, has required other approaches for estimating the 2^1A_g energies of these molecules. In particular, the weak-coupling limit of the energy-gap law developed by Englman and Jortner (1970) appears to be well suited for describing $S_1 \rightarrow S_0$ nonradiative decay (internal conversion) in long polyenes: $E(S_1) - E(S_0) = \Delta E$ is large and internal conversion is dominated by a single type of vibrational accepting mode, i.e., symmetric C=C stretches. A simplified version of this model is summarized as follows:

$$k_{ic} = C \exp(-\gamma\Delta E/h\nu) \quad \text{Eq. (3)}$$

where k_{ic} is the rate of $S_1 \rightarrow S_0$ internal conversion (in most cases the reciprocal of the S_1 lifetime), ΔE is the energy difference between the S_0 and S_1 states, and $h\nu$ is the energy of the S_0 accepting vibrations ($\sim 1600 \text{ cm}^{-1}$). C and γ are assumed to have only a mild dependence on ΔE , $h\nu$, the number of accepting modes, and the displacement between the S_0 and S_1 potential energy surfaces. C , γ , and $h\nu$ often are treated as constants in fits relating k_{ic} to ΔE for different carotenoids. (See Chynwat and Frank (1995) and Andersson et al. (1995) for further discussion of these assumptions.) These simplifications lead to a linear version of the energy-gap law:

$$\ln k_{ic} = \ln C - B\Delta E \quad \text{Eq. (4)}$$

Andersson et al. (1995) applied the energy-gap

law to a series of carotenes, Frank et al. (1993) and Chynwat and Frank (1995) carried out a similar study on spheroidenes and other carotenoids, and Bachilo et al (1998) studied S_1 internal conversion rates in diphenylpolyenes. The rates of S_1 nonradiative decay (determined from fluorescence lifetime or transient absorption measurements) and the $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ (0-0) transition energies (determined (or estimated) from spectroscopic measurements) appear to obey the energy-gap law, e.g., plots of $\ln k_{ic}$ vs. ΔE tend to obey equation 4 with $\ln C \sim 35-40$ and $1/B (= h\nu/\gamma) \sim 1100-1500 \text{ cm}^{-1}$ for a wide range of polyenes and carotenoids (Frank et al., 1993; Andersson et al., 1995; Bachilo et al., 1998). More sophisticated applications of the energy-gap law have been discussed by Chynwat and Frank (1995). Parameters obtained from molecules for which both ΔE and k_{ic} are known then are used to extrapolate $\Delta E = E(2^1A_g)$ for longer carotenoids for which only k_{ic} can be experimentally determined. This approach has been used to estimate the 2^1A_g energies of several nonfluorescent carotenoids that play important roles in photobiological systems (Frank et al., 1993, 1997; Chynwat and Frank, 1995).

The value of energy gap extrapolations depends both on the applicability of the model to the range of molecules being considered and the quality of data used to determine the empirical relationships between k_{ic} and ΔE . One of the most fundamental limitations in applying energy gap analysis to carotenoids can be traced to unresolved optical spectra and the somewhat arbitrary assignment of ΔE values in various solvents. Andersson et al. (1995) obtained S_1 lifetimes of a series of molecules homologous to β -carotene, and $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ (0-0)'s (ΔE 's) were based on fluorescence spectra obtained in 77 K, 3-methylpentane glasses (Andersson et al., 1992). Even under low temperature conditions, the carotene spectra are essentially unresolved. The (0-0) bands for $S_0 \rightarrow S_2$ and $S_1 \rightarrow S_0$ transitions only could be estimated from absorption maxima, with errors of $\pm 1000 \text{ cm}^{-1}$ reported for the mini-5, mini-7, and mini-9 (0-0) transitions (Andersson et al., 1992). In a recent application of the energy-gap law to this series, Andersson et al. (1995) use S_1 depopulation rates in room temperature hexane and Eq. (4) to extrapolate the S_1 energies for two longer, nonfluorescent carotenoids (with $N = 15$ and $N = 19$) whose S_1 lifetimes were obtained by transient absorption techniques. The 77 K mini-carotene $S_1 \rightarrow S_0$ transition

energies were systematically lowered by 1000 cm^{-1} to estimate the ΔE 's in room temperature hexane. This adjustment plus the uncertainties in locating the (0-0)'s in the 77 K glasses give rise to considerable uncertainties in the ΔE 's used to determine the parameters in Eq. (4).

The energy gap studies of Frank et al. (Chynwat and Frank, 1995; Frank et al., 1997) are based on $S_1 \rightarrow S_0$ fluorescence spectra and S_1 lifetime measurements of three synthetic spheroidenes (DeCoster et al., 1992), two mini-carotenes (Andersson and Gillbro, 1992), and fucoxanthin (Kato et al., 1991; Shreve et al., 1991; Mimuro et al., 1992). Problems regarding the uncertainties in the 2^1A_g (0-0) energies are partially overcome, e.g., the vibronic resolution is sufficient to locate $S_1 \rightarrow S_0$ electronic origins in the two shorter spheroidenes and in fucoxanthin. However, the (0-0) energies must be estimated for the other three molecules, including two of the mini-carotenes discussed above. Lifetimes ($\sim 1/k_{ic}$) were obtained in diethylether for the spheroidenes, CS_2 for fucoxanthin, and n-hexane for the mini-carotenes. Corresponding ΔE 's were obtained in methanol, CS_2 and 77 K 3-methylpentane glasses (Andersson et al., 1992). Unlike Andersson et al. (1995), Frank et al. retain the ΔE 's estimated from the original 77 K spectra of the two mini-carotenes and relate these energies to lifetimes obtained in room temperature solutions. Future applications of the energy-gap law to estimate S_1 energies in nonfluorescent carotenoids should carefully consider how k_{ic} and ΔE (and the parameters obtained from Eqs. (3) and (4)) depend both on solvent and on temperature.

Another consideration is the appropriateness of applying energy-gap law fits to molecules with significantly different structures. Whereas the studies by Andersson et al. were confined to a homologous series, Frank et al. employed a range of carotenoids in their extrapolations. A survey of recent energy-gap law fits to spheroidenes (Frank et al., 1993), carotenes (Andersson et al., 1995), and diphenyl polyenes (Bachilo et al., 1998) shows systematic differences in parameters (B and C in Eq. (4)) which may be related to differences in molecular structure. Furthermore, a previous study of the gas phase fluorescence of simple tetraenes and pentaenes showed significant increases in $S_2 \rightarrow S_1$ internal conversion rates upon methyl-substitution where the $E(S_2) - E(S_1)$ difference remained constant (Bouwmans et al., 1990). The acceleration of internal conversion was attributed to the increased density of

S_1 vibronic states in methyl-substituted compounds. Similar effects may modify $S_1 \rightarrow S_0$ internal conversion rates in carotenoids, e.g., B and C in equation 4 may be different for carotenes and spheroidenes and/or depend on the length of conjugation. Typical parameters ($1/B \approx 1100\text{--}1500\text{ cm}^{-1}$) indicate that changing k_{ic} by a factor of two (e.g., by modifying the solvent, temperature, or molecular structure) changes ΔE by $750\text{--}1050\text{ cm}^{-1}$. This argues for caution in applying the energy-gap law to a wide range of conjugated molecules under different solvent conditions, even if k_{ic} and ΔE both can be accurately determined. These limitations should cause particular pause in using lifetime-based estimates of 2^1A_g energies to extrapolate to infinite carotenes or spheroidenes (Table 1).

4. Recent Attempts at the Direct Detection of the 2^1A_g State in β -Carotene and Other Long Carotenoids

Due largely to its photobiological importance and the availability of high purity samples, β -carotene has been a popular target for the initial application of a wide variety of spectroscopic techniques to elucidate the energies and properties of 'long carotenoid' electronic states. Recent publications include the observation of a relatively strong absorption at $14,200\text{ cm}^{-1}$ in zeolite, detected by reflectance spectroscopy (Haley et al., 1992). The authors argue that the symmetry-forbidden, $g \rightarrow g$ transition is made allowed by distortion of β -carotene by asymmetric sites in the zeolite host. However, a $14,120\text{ cm}^{-1}$ peak in the reflectance spectrum of a 4.2 K single crystal of β -carotene was attributed to an experimental artifact (Gaier et al., 1991). In this same study, Gaier et al. reported low energy features in the pre-resonance Raman excitation spectra of β -carotene single crystals that were assigned to $S_0(1^1A_g) \rightarrow S_1(2^1A_g)$ absorption. Analysis of these bands leads to an $13,600\text{ cm}^{-1}$ estimate for the (0-0). Another intriguing result was the observation of a weak absorption background in the inverse Raman (Raman loss) spectrum of canthaxanthin (Jones et al., 1992) which shows evidence for a low lying absorption at $\sim 600\text{--}700\text{ nm}$. However, there is little support for the identification of this feature with the $S_0(1^1A_g) \rightarrow S_1(2^1A_g)$ transition. Rohlifing et al. (1996) have investigated the electric-field-induced change in the absorption (electroabsorption) of β -carotene and a model octaene in polystyrene matrices. Electroabsorption is directly

related to the third-order nonlinear susceptibilities of these molecules. A weak, low-energy feature in the electroabsorption spectrum of the octaene at 2.65 eV ($21,400\text{ cm}^{-1}$) was associated with $1^1A_g \rightarrow 2^1A_g$ absorption activated by the symmetry-breaking effect of the applied electric field. A similar but weaker response was reported for β -carotene. All of the studies on β -carotene would benefit from more systematic extensions to shorter polyenes/carotenoids for which the energies of the 2^1A_g states can be unambiguously established by detection of vibronically resolved, $S_1 \rightarrow S_0$ fluorescence. This would allow straightforward evaluation of the ability of these alternate techniques to detect $1^1A_g \rightarrow 2^1A_g$ transitions in molecules such as β -carotene.

The most promising of recent efforts to detect the 2^1A_g state in long carotenoids began with the report by Bondarev and Knyukshto (1994) of a very weak ($\phi_f \sim 10^{-5}$) and very broad, $S_1 \rightarrow S_0$ emission in β -carotene. These authors somewhat arbitrarily assigned the $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ (0-0) energy as $13,200 \pm 300\text{ cm}^{-1}$ in *n*-hexane, toluene, and carbon disulfide. Andersson et al. (1995) later repeated the results of Bondarev and Knyukshto but reported sufficient vibronic structure in carbon disulfide to place the (0-0) transition at $14,200\text{ cm}^{-1}$. However, Andersson et al. did not present fluorescence excitation spectra, a critical issue in proving that the weak, long-wavelength emissions belonged to β -carotene. Koyama and Fujii (Chapter 9) and Fujii et al. (1998) recently extended these earlier studies to detect weak, $S_1(2^1A_g) \rightarrow S_0(1^1A_g)$ (0-0) transitions in *all-trans* isomers of β -carotene ($14,500\text{ cm}^{-1}$), spheroidene ($14,200\text{ cm}^{-1}$), and neurosporene ($15,300\text{ cm}^{-1}$) in *n*-hexane. These assignments are supported by fluorescence excitation spectra that are in excellent agreement with the $S_0 \rightarrow S_2$ absorption spectra. The locations of the (0-0) bands confirm previous estimates (Cosgrove et al., 1990; Andersson et al., 1992; DeCoster et al., 1992; Frank et al., 1993, 1997; Chynwat and Frank, 1995) based on extrapolations from shorter, more fluorescent analogs.

These recent experiments demonstrate that it should be possible to use fluorescence to detect resolved, $S_1 \rightarrow S_0$ emissions in other long carotenoids, although such studies will require samples of high purity and should include a careful analysis of fluorescence excitation spectra to confirm the source of any weak, low energy emissions. The extremely low $S_1 \rightarrow S_0$ fluorescence yields ($<10^{-3}$ for molecules such as β -carotene) put heavy demands both on the quality of

the samples and the interpretation of the experiments in order to exclude the possibility of interferences from other emissive species. Thus, for example, the original claims of 2^1A_g emissions in spheroidene (Watanabe et al., 1993) later were showed to be due to chlorophyll *a* impurities in the spheroidene preparations (Koyama et al., 1996; Frank et al., 1997; Fujii et al., 1998).

Finally, it is important to comment on the very recent report (Sashima et al. (1998)) of the detection of the 2^1A_g state in solid *all-trans*-spheroidene using resonance Raman excitation techniques. The potential of using resonance Raman excitation spectra to detect resolved $S_0 \rightarrow S_1$ absorptions in carotenoids provides a tantalizing alternative to the detection of weak fluorescence signals and/or the use of extrapolation techniques (energy gap and other approaches). However, the application of resonance Raman excitation techniques to carotenoids has a tortuous past. See DeCoster et al. (1992) and Frank and Christensen (1995) for a discussion of previous attempts to use Raman excitation techniques to locate the S_1 state in β -carotene, starting with the early reports of Thrash et al. (1977, 1979). It thus will be important to see if the experiments reported by Sashima et al. on spheroidene can be readily extended to shorter polyenes and carotenoids for which the $S_0 \leftrightarrow S_1$ (0-0)'s have been unambiguously located by fluorescence techniques. Similar features in the resonance Raman excitation profiles of 4.2 K single crystals of β -carotene (Gaier et al., 1991) were only cautiously assigned to $S_0 \rightarrow S_1$ absorptions. The vibronic progressions in the excitation profiles of Sashima et al. appear to depend on the vibrational mode monitored (C-C or C=C symmetric stretch), whereas the excitation profiles of Gaier et al. do not depend on the Raman mode monitored. It also should be noted that the excitation profiles reported by Gaier et al. and by Sashima et al. both show maximum intensities in what are identified as the (0-0) and (0-1) bands of the $S_0 \rightarrow S_1$ transitions. This is in stark contrast to the steep, monotonic rises in $S_0 \rightarrow S_1$ vibronic intensities observed in high resolution spectra of model polyenes (Simpson et al., 1987; Kohler et al., 1988; Petek et al., 1991). To the extent that the forbidden, $S_0 \rightarrow S_1$ transitions are made 'allowed' by vibronic interactions between 2^1A_g and 1^1B_u , $S_0 \rightarrow S_1$ (0-0) bands are typically rather weak compared to vibronic transitions to S_1 vibrational states that are closer to the zero-point energy of $S_2(1^1B_u)$. A careful examination of relative vibronic

intensities thus should be part of any assignment of low-energy features in carotenoid electronic spectra.

IV. Triplet States in Polyenes and Carotenoids: Spectroscopic Observations and Theory

Compared to excited singlet states, considerably less experimental information is available regarding the energies of polyene/carotenoid triplet states. This, in large part, is due to their lack of confirmed phosphorescence. This presents a major barrier to understanding the energies and properties of triplets, particularly of longer polyenes and of the carotenoids involved in photobiological processes. Evans' pioneering experiments using high pressure, oxygen perturbation techniques to enhance $S_0 \rightarrow T_1$ absorption spectra (1960, 1961, 1972) provided the first direct observations of low-lying triplet states in ethylene, butadiene, hexatriene, and octatetraene. Evans (1960) also detected $S_0 \rightarrow T_1$ transitions in octatrienal, decatetraenal, and dodecapentaenal. This work was extended to retinal by Raubach and Guzzo (1973), though their assignment of the 803 nm band as the $S_0 \rightarrow T_1$ electronic origin probably is in error. (Evans' work on dodecapentaenal indicates that the (0-0) band for retinal most likely is at ~900 nm.) Electron-impact spectroscopy (Kuppermann, 1979; Allan et al. 1984) later provided complementary information on the low-lying excited triplet states of the short polyenes. The results of these measurements and the gas-phase measurements of the $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions in these simple polyenes are summarized in Table 2.

There is strong theoretical support for assigning the lowest lying triplet state in polyenes (T_1) as 3B_u (Allen et al. 1984; Zoos and Ramasesha 1983). It is

important to note the distinctions between data obtained in solution versus that obtained in the gas-phase and whether there is sufficient resolution to allow the unambiguous identification of electronic origins as opposed to estimates of the Franck-Condon maxima of 'vertical' transitions. Data obtained by electron-impact and oxygen perturbation techniques are in excellent agreement. This not only provides confidence in the measurements, but also confirms that the spin-forbidden, $S_0 \rightarrow T_1$ transitions are relatively insensitive to solvent perturbations. It is significant that very little additional spectroscopic information regarding polyene triplets has been obtained since the early work of Evans. True to form, octatetraene provides the most accurate, most complete set of data for the singlet and triplet electronic energies of any polyene. The vibronic features of all $S_0 \rightarrow T_1$ spectra are very similar to those observed for corresponding $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions. Vibronic intervals of 1400–1500 cm^{-1} can be traced to unresolved combinations of C=C and C-C symmetric stretching vibrations. $S_0 \rightarrow T_1$ (0-0) bands are typically weak, suggesting a more significant change in geometry than for low energy $S \rightarrow S$ transitions. The characteristic vibronic signatures of polyene spectra again provide an useful tool for assigning electronic transitions.

Little, if any reliable spectroscopic information is available regarding the T_1 energies of polyenes or carotenoids with more than six conjugated double bonds. As a result, it has been necessary to rely on indirect measurements and/or extrapolations to estimate the T_1 energies of carotenoids employed in photosynthetic systems. One such approach has been outlined by Bensasson et al. (1976, 1993) and discussed by Frank and Cogdell (1993). A plot of the reciprocal of the triplet energies (from Table 2) as a

Table 2. Energies (in cm^{-1}) of low-lying electronic states of simple polyenes. Vertical transition energies (Franck-Condon maxima) are given in parentheses. Other energies refer to the electronic origins (0-0 bands) of $S_0 \rightarrow T_1$ and $S_0 \rightarrow S_n$ transitions. The O_2 perturbation spectra were obtained in chloroform; all other energies refer to the gas phase.

	O_2 perturbation ^a ${}^1{}^3B_u$	electron impact ^b ${}^1{}^3B_u$	$2{}^1A_g$	$1{}^1B_u$
ethylene	<28,700 (37,000)	(34,800)		~48,000 ^f
butadiene	20,830 (26,000)	(26,000)	~43,500 ^c	46,260 ^g
hexatriene	16,450 (20,800)	(21,000)	<34,038 ^d	39,786 ^g
octatetraene	13,750 (17,000)	13,900 (16,900)	29,949 ^e	35,553 ^g

^a Evans (1960,1961); ^b Allan et al. (1984); Kuppermann et al. (1979); ^c Chadwick et al. (1985); ^d Buma et al. (1990); ^e Petek et al. (1995); ^f Ziegler et al. (1983); ^g Vaida (1984)

function of the number of double bonds gives a linear fit that has been used to extrapolate to the triplet energies of more extensively conjugated molecules such as spheroidene and β -carotene. There is little justification for extrapolating from $N = 4$ to $N = 11$. Nevertheless, this procedure (or a plot of triplet energies vs. N^{-1} , cf. Table 1) predicts T_1 energies for longer carotenoids, e.g., ~ 6200 – 6800 cm^{-1} for β -carotene, depending on assumptions regarding the effective length of conjugation (Bensasson, 1976, 1993). These energies are consistent with those estimated from quenching experiments in which molecules of known triplet energy are used to sensitize the formation of β -carotene triplets. Detailed quenching experiments, particularly involving the quenching of singlet oxygen (Bensasson, 1993), place the T_1 level of β -carotene to be almost isoenergetic with that of excited, singlet oxygen (~ 7900 cm^{-1}).

Extensive use also has been made of the approximation from the simple valence bond description of polyene energy levels that the lowest triplet level in polyenes/carotenoids (1^3B_u) should have an energy approximately one half that of the lowest singlet state (2^1A_g) (Hudson et al., 1982). The limited data given in Table 2 indicates that this relationship is approximately true for short polyenes. Though there is only qualitative theoretical support for this statement, valence bond theory has yet to provide a quantitative accounting of the energies of low-lying singlet states in long or short polyenes., the lack of alternate spectroscopic information has allowed the $(E(1^1A_g \rightarrow 1^3B_u) = E(1^1A_g \rightarrow 2^1A_g)/2)$ 'rule of thumb' to find wide use in estimating the energies of carotenoid triplets. (See, for example, Haley et al 1992) As discussed in section III, recently improved estimates of the 2^1A_g energy in β -carotene (Cosgrove et al. 1990; Frank et al., 1993, 1997; Bondarev and Knyukshto 1994; Andersson et al. 1995, Koyama and Fujii, Chapter 9) converge on an 2^1A_g energy of $14,500 \pm 500$ cm^{-1} . The rule of thumb leads to a 1^3B_u (T_1) energy of $\sim 7,250$ cm^{-1} which is consistent with the results of quenching experiments and the extrapolation of Evans' data on short polyenes.

Bachilo (1995) recently used transient absorption techniques to detect a weak $T_1 \rightarrow T_n$ transition at 8100 cm^{-1} in β -carotene. This places T_n (3B_u or 3A_g ?) at almost the same energy as S_1 . Assuming (as appears to be the case for shorter polyenes (Allan et al. 1984)) that the lowest triplet state is 3B_u and that there is a nominal splitting between 2^1A_g and its corresponding triplet, there must be an undetected

3A_g state lying somewhere between T_1 ($1^3B_u \sim 7000 \pm 1000$ cm^{-1}) and S_1 ($2^1A_g \sim 14,500$ cm^{-1}). Understanding the energies and symmetry labels of these low-lying triplet states will be important in more fully describing spin-orbit coupling and triplet quenching in this well-studied molecule.

Marston et al. (1995) reported the detection of phosphorescence from β -carotene in the near IR using Fourier transform techniques, placing T_1 at ~ 7400 cm^{-1} . This study would have benefited by systematic application to longer and shorter conjugated systems, the time-honored way to demonstrate that the energies of carotenoid excited states depend inversely on the length of conjugation. Furthermore, the phosphorescence spectrum reported by Marston shows vibronic structure with ~ 500 cm^{-1} spacings. This is not consistent with the higher frequency C-C and C=C stretches that dominate polyene singlet \leftrightarrow singlet spectra (Section II.B.1 and Fig. 3) as well as the vibronic patterns observed in the $S_0 \rightarrow T_1$ transitions of short polyene hydrocarbons (Evans 1960, 1961; Allan et al. 1984).

In contrast to the wide-ranging efforts to detect triplet states in β -carotene, very little effort has been expended on other carotenoids such as spheroidene. Our understanding of low-lying triplet energies in these systems is limited to quenching experiments (Farhoosh et al., 1994, 1997), estimates based on extrapolations from the triplet energies of shorter polyenes, and the use of the $E(1^3B_u) = E(2^1A_g)/2$ approximation. It is important to reiterate that the use of extrapolations or the rule-of-thumb requires critical scrutiny, especially for carotenoids for which this is the only means of estimating triplet energies.

V. Conclusions and Unresolved Issues

The primary focus of this chapter has been the connection between the spectroscopy of simple polyenes and the low-lying, excited electronic states of carotenoids involved in photobiological processes. A survey of recent work indicates the crucial significance of 'high-resolution' spectroscopic experiments: first, in establishing the existence of low energy 2^1A_g states in model systems and subsequently, in understanding how the energies of these states change with increasing conjugation, the presence of substituents, and different solvent environments. The simple geometries of unsubstituted molecules allow their incorporation into low

temperature mixed crystals, and the relatively high vapor pressures of short polyenes permit their detailed study as low-temperature, isolated molecules in supersonic jets. These experiments (resolutions of $<1\text{ cm}^{-1}$ in supersonic jets, $<10\text{ cm}^{-1}$ in n-alkane crystals) and, to a lesser extent, experiments carried out in low temperature glasses (resolutions of $>100\text{ cm}^{-1}$, e.g., see Figs. 4 and 5), provide sufficient vibronic resolution to allow the unambiguous identification of electronic origins as well as a detailed look at other vibrational states accessed by both symmetry-allowed and symmetry-forbidden electronic transitions.

The major challenges in extending the experiments on short polyenes to the longer, more complicated carotenoids employed in photobiology are the precipitous decreases in $S_1 \rightarrow S_0$ fluorescence yields combined with the losses in spectral resolution. (High-resolution mixed crystal and gas phase techniques are not easily applied to molecules such as β -carotene.) The limited experimental data available for longer carotenoids and the subsequent need for ad hoc extrapolations leads to 'low-resolution' estimates of the 2^1A_g energies (uncertainties of hundreds of cm^{-1}), in contrast to the much higher precisions to which electronic and vibrational energies can be determined for shorter polyenes. On the other hand, such low-resolution estimates may be quite sufficient for understanding the mechanisms of energy transfer in photosynthetic systems. For example, it now seems clear that the 2^1A_g state of spheroidene ($E \sim 14,200\text{ cm}^{-1}$ in n-hexane) lies well above the $S_1(Q_1)$ state of bacteriochlorophyll *a* (e.g., $E \sim 12,500\text{ cm}^{-1}$ in the B800 monomer of the light harvesting complex (LH2) of *Rhodobacter sphaeroides* (Sauer, 1978)), allowing efficient spheroidene (S_1) \rightarrow bacteriochlorophyll *a* (S_1) energy transfer in the antenna complexes of typical photosynthetic bacteria.

In other situations, more accurate estimates of carotenoid electronic energies may be required. The $S_1(2^1A_g)$ state of β -carotene ($E \sim 14,500\text{ cm}^{-1}$ in n-hexane) appears to lie somewhat below the $S_1(Q_1)$ state of chlorophyll *a* (e.g., $E \sim 14,700\text{ cm}^{-1}$ in the light-harvesting complex of Photosystem II (Kwa et al. 1992)). This suggests that the S_2 state of β -carotene must be involved in energy transfer (Cosgrove et al., 1990; DeCoster et al., 1992; Frank and Christensen, 1995), as originally proposed by Snyder et al. (1985). However, more accurate estimates of the in vivo $S_1(2^1A_g)$ energies of β -carotene are required to fully understand the relative roles of the carotenoid S_2 and

S_1 states in a variety of photosynthetic antennae. Similarly, one attempt to explain how excess energy is dissipated by plants under conditions of high photon fluxes relies on subtle ($\sim 500\text{--}1000\text{ cm}^{-1}$) differences between the 2^1A_g energies of three xanthophylls (violaxanthin ($N = 9$), antheraxanthin ($N = 10$), and zeaxanthin ($N = 11$)) found in the light-harvesting machinery of plants (Frank et al., 1994). More reliable estimates of 2^1A_g energy differences in biological environments would be helpful in understanding the interactions between the xanthophylls and chlorophyll. Note also that the population of low frequency vibrations and phonons results in a significant fraction of states ($\sim e^{-\Delta E/kT}$) within a few kT ($\sim 200\text{ cm}^{-1}$ at 25°C) of the electronic origins. Thermal effects thus need to be considered in understanding the interactions between closely separated carotenoid and chlorophyll excited states. This also suggests that kT might be an appropriate experimental goal for the accuracy of electronic energy determinations of carotenoids employed in photobiological processes.

In spite of the inherent difficulties in locating 2^1A_g states in weakly fluorescent samples, no better approach has emerged that yields unambiguous assignments of $S_0 \leftrightarrow S_1$ electronic origins in long polyenes or carotenoids. Two-photon spectroscopy, reflectance spectroscopy, resonance Raman excitation spectroscopy, consecutive two-photon absorption, electroabsorption, etc. have failed to improve on (or, in many cases, even to reproduce) data obtained from fluorescence experiments on shorter carotenoids and polyenes. A fundamental problem with techniques based on one-photon absorption (i.e., almost all the techniques listed above) is the difficulty in locating the extremely weak $S_0 \rightarrow S_1$ (0-0) bands in other than high resolution experiments. Polyene $1^1A_g \rightarrow 2^1A_g$ absorptions show steep rises in vibronic intensities in approaching the $1^1A_g \rightarrow 1^1B_u$ origins due to a $(\Delta E)^{-2}$ dependence on vibronic mixing/intensity borrowing between the 2^1A_g and 1^1B_u states (Petek et al., 1991). This effect is particularly evident in high-resolution spectra of model heptaenes and octaenes (Simpson et al., 1987; Kohler et al., 1988). The current lack of suitable spectroscopic alternatives means that extrapolation procedures, e.g., the use of the energy-gap law to estimate 2^1A_g energies in nonfluorescent carotenoids, will continue to be used. Extrapolations, including the fitting of experimental energies to various functions of N , would very much benefit from a careful analysis of uncertainties in the original data (including corrections for solvent

perturbations) and the propagation of these errors into estimates of 2^1A_g energies for larger N .

Outstanding issues to be addressed by more accurate information on the electronic energies of long polyenes and carotenoids include a quantitative understanding of the differences between the $S_0 \leftrightarrow S_1$ and $S_0 \leftrightarrow S_2$ transition energies of simple polyenes, spheroidenes, and carotenoids. Although a relevant comparison for $N=7$ is provided by Fig. 4, more comprehensive studies are needed for larger N to understand the effects of conjugation length and substituents (both methyl groups and terminal β -cyclohexenyl rings) on S_1 and S_2 energies. An interesting sidelight of these studies will be a better understanding of the effects of substitution on S_1 and S_2 energies in the long/infinite polyene limit and the resolution of the discrepancies summarized in Table 1.

Our understanding of the effects of solvents on S_1 energies is limited to the application of Eqs. (1) and (2) to the transition energies of short, model polyenes. A systematic look at both $S_0 \leftrightarrow S_1$ and $S_0 \leftrightarrow S_2$ solvent shifts (i.e., the determination of k 's in Eqs. (1) and (2)) in longer carotenoids would be useful in exploring systematic changes as a function of conjugation length. This not only would be helpful in evaluating and comparing transition energies obtained in different solvents (including biological matrices), but also will be essential in allowing more accurate extrapolations of transition energies to infinite polyenes and carotenoids.

Any extension of the spectroscopic investigations of energy levels naturally should be coupled with systematic investigations of S_1 and S_2 dynamics to develop a better understanding of substituent effects and isomeric structure on radiative and nonradiative processes. Such studies would include more detailed tests of the energy-gap law for specific homologous series and quantitative investigations of differences in the rates of radiationless processes due to details of molecular structure, e.g., the differences between spheroidenes and carotenes. Recent studies on spheroidene (Ricci et al., 1996) and β -carotene (Macpherson and Gillbro, 1998) show that their S_2 internal conversion rates are strongly dependent on solvent. Parallel investigations of the effect of solvent environment (including temperature) on S_1 dynamics also will be important in understanding the interplay between energetics and solvent effects in determining the rates of radiative and nonradiative decay.

The spectroscopic catalog of polyene T_1 triplet energies (Table 2) has expanded very little since the

early 1960s (Evans, 1960, 1961). Given the importance of these states (and their energies) to the protective function of carotenoids and the increase sophistication of spectroscopic techniques, it is remarkable that so little new information is available concerning triplet energies in longer polyenes or carotenoids. The $E(T_1) \sim E(S_1)/2$ rule-of-thumb is based almost entirely on data from simple polyenes with $N=1-4$. A systematic look at longer polyenes is needed to refine and extend this approximation to carotenoids of biological significance.

Kohler and Samuel (1995) recently examined the absorption spectra of long, synthetic polyenes ($N=20-240$) and noted that these molecules absorb at wavelengths significantly shorter than those predicted by the extrapolation of spectra of shorter, model systems (Table 1). Effects of conformational disorder were invoked to explain the spectra, leading to a distinction between 'conjugation length' and 'chain length' in long polyenes. According to this model (Kohler and Woehl, 1995), twists about polyene single bonds break the chain into a distribution of shorter polyene segments with statistical (entropy) considerations predicting a dominance of short conjugation length segments in room temperature solutions. The analysis of these effects strongly suggests that conformational disorder also should be important for polyenes of intermediate length, including naturally occurring carotenoids. It is important to stress that there currently is no evidence for such effects in the spectroscopy of short polyenes or the carotenoids discussed in this review. Nevertheless, conformational disorder would have far-reaching implications for understanding the spectroscopy and photochemistry of carotenoids, and this hypothesis warrants careful consideration.

This chapter has focused almost exclusively on the electronic structures and dynamics associated with carotenoids in *all-trans* configurations. Although *all-trans* carotenoids often are selected by light-harvesting complexes, *cis*-isomers tend to be employed by photosynthetic reaction centers, and it is important to understand both how the energetics and the kinetics are modified for *cis* conformers. One obvious problem in carrying out such studies, at least in vitro, is the thermal and photochemical instability of the *cis* forms. Nevertheless, the importance of these isomers in vision and in photoprotection provides strong motivation for extending spectroscopic and time-resolved studies to these systems.

This chapter has adopted the approach that all of

the singlet state photochemistry of *cis* and *trans* carotenoids can be explained using the energy level diagram presented in Fig. 1. It is important to mention that there is both experimental and theoretical support for the incursion of other electronic states into the low energy regions of this figure. For example, nonlinear optical measurements on *all-trans*- β -carotene indicate the existence of a 1A_g state ~ 1000 cm^{-1} above the 1^1B_u state (van Beek et al., 1992). In addition, the PPP-MRD-CI calculations of Tavan and Schulten suggest the presence of another low-lying 'covalent' state ($^1B_u^-$) which for $N > 5$ lies below the 'ionic' 1^1B_u state ($^1B_u^+$) identified as S_2 throughout this review. Although there is at present very little experimental support for additional, electronic levels ($^1B_u^-$) or otherwise) with energies less than $E(S_2)$, improvements in spectroscopic techniques eventually should allow a more rigorous search for these states in longer carotenoids.

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