

quence the spectroscopic characteristics of violaxanthin are expected to be very similar to those of the open-chain carotenoids studied here.

In this work, a spectroscopic study of three open-chain, *all-trans*-C₃₀ carotenoids is presented. These molecules are derived from spheroidene, the major carotenoid from the photosynthetic bacterium, *Rhodobacter (Rb.) sphaeroides* wild type, but lack the methoxy-functional group and the nonconjugated, floppy, C₁₀-terminal alkyl group of the parent molecule. The rationale for studying these derivatives is that without the substituents, higher resolved optical spectra may be observed that would facilitate the assignment of the positions of the excited-state energy levels. The structures of the C₃₀ carotenoids, 2,6,10,15,19,23-hexamethyl-2,6,8,10,12,14,16,18,22-tetracosanonaene, 2,6,10,15,19,23-hexamethyl-2,6,8,10,12,14,16,18,20-tetracosanonaene, and 2,6,10,15,19,23-hexamethyl-4,6,8,10,12,14,16,18,20-tetracosanonaene are shown in Figure 1 and are hereafter are referred to as heptaene, octaene, and nonaene to indicate the number of double bonds in conjugation. The samples were purified by high performance liquid chromatography (HPLC) and studied using steady-state absorption, fluorescence, fluorescence excitation and time-resolved absorption spectroscopy. The open-chain analogues exhibit characteristics that are important for understanding the photochemical properties of carotenoids and polyenes in general, and for elucidating the specific molecular features that control the ability of their more highly conjugated counterparts to function in photosynthesis.

Materials and Methods

Synthesis. A description of the synthesis of the heptaene and nonaene molecules has already been published.³⁷ To prepare the C₃₀ diapocarotene with eight conjugated double bonds, isovaleraldehyde (3-methylbutanal, Aldrich) was coupled to a C₅-phosphonate ester³⁸ in a Horner–Wadsworth–Emmons (HWE) reaction. The resulting ester was reduced to the corresponding C₁₀ alcohol with Dibal-H. The C₁₀ alcohol then was converted into a phosphonium salt by reacting with triphenylphosphonium hydrobromide, and coupled in a Wittig reaction to a C₂₀ aldehyde that was prepared as follows: Geraniol (Aldrich) was oxidized with MnO₂ to yield geranial, which was coupled in a HWE reaction to a C₅-phosphonate nitrile.³⁹ The resulting nitrile was reduced with Dibal-H to the corresponding C₁₅ aldehyde. This aldehyde was coupled in a

HWE reaction to another C₅-phosphonate nitrile^{40–42} to yield, after Dibal reduction, the C₂₀ aldehyde.

Sample Preparation. The carotenoids were purified using a Millipore Waters 600E HPLC employing a 5 μm YMC C₃₀ carotenoid column (4.6 mm × 250 mm). The mobile phase was 11:89 v/v methyl *tert*-butyl ether (MTBE)/methanol for the heptaene and the octaene and 38:62 v/v MTBE/methanol for the nonaene. The runs were isocratic at 1.0 mL/min. The heptaene, octaene and nonaene absorption bands were monitored at 400, 419 and 436 nm, respectively, using a photodiode array as a detector. The *all-trans* isomers were collected and dried with a gentle stream of nitrogen. The samples were kept in the dark until ready for use. EPA (ether/isopentane/ethanol, 5/5/2, v/v/v) was added to the samples prior to taking the absorption and fluorescence spectra at 293 and 77 K.

Spectroscopic Methods. Steady-State Absorption and Fluorescence. Absorption spectra of the samples in *n*-hexane and EPA solutions at 293 K and in EPA glasses at 77 K were recorded on a Cary 50 UV spectrometer. Absorption spectra were obtained from the samples in 4 mm (interior width) Suprasil square quartz cuvettes suspended in a cylindrical custom-made (Kontes) liquid nitrogen cryostat that was either empty for the experiment at 293 K or filled with liquid nitrogen for the experiment at 77 K. A gentle stream of He gas bubbled near the cuvette minimized agitation of the liquid nitrogen.

Fluorescence spectra were obtained using the same cuvette and cryostat used in absorption experiments. The fluorescence experiments were carried out using an SLM Instruments, Inc. Model 8000C spectrofluorometer, and the emission was detected by a Hamamatsu R928 photomultiplier (PMT) tube. An SLM Instrument Model WCTS-1 thermostatically cooled housing was used to reduce the PMT dark current. For fluorescence excitation spectroscopy the spectral profile of the incident light was monitored using HITEC perchlorate as a quantum counter. A 450 W ozone free OSRAM XBO xenon arc lamp was used to excite the heptaene and octaene. The 463 or 477 nm lines from a

with 16 nm band-pass for both excitation and emission monochromators. A 470 nm long-pass cutoff filter was used for 293 K experiment, and a 460 nm cutoff filter was used for the 77 K experiment.

The nonaene was excited using an argon ion laser at 463 nm for the 293 K experiment and at 477 nm for the 77 K experiment. A 495 nm long-pass cutoff filter was used for both

HPLC protocol also removed impurities eluting at short ($\sim 3\text{--}8$ min) retention times. These molecules absorb at <400 nm and are most likely less conjugated degradation products of the carotenoids. It was essential that these polyene impurities be removed because they have relatively high fluorescence yields and can adversely affect the fluorescence and the fluorescence excitation spectra.

Absorption and Fluorescence Spectra. The absorption spectra of the molecules in EPA at 77 and 293 K are shown in Figure 3 and are observed to red-shift with increasing extent of the π -conjugation. The full width at half-height of the 0–0

with increasing extent of π -electron conjugation. The quantum

are remarkably insensitive to conjugation length. For example, Andersson et al.²⁹

is confirmed by the $S_1 \rightarrow S_0$ quantum yields given in Table 3. The $S_1 \rightarrow S_0$ quantum yields decrease by roughly 2 orders of magnitude with increasing conjugated chain length from seven to nine conjugated carbon-carbon double bonds. The $S_2 \rightarrow S_1$ internal conversion rates (and $S_2 \rightarrow S_0$ fluorescence yields) are relatively constant for these molecules, and the sharp decrease in $S_1 \rightarrow S_0$ fluorescence accounts for the increase in the ratio of $S_2 \rightarrow S_0/S_1 \rightarrow S_0$ emission.

The prominent vibronic bands for the $S_1 \rightarrow S_0$ transitions are given in Tables 1 and 2. The (0-0), (0-1), (0-2), etc., notation refers to unresolved progressions of the totally symmetric C-C and C=C stretching modes that dominate the Franck-Condon envelopes of polyene/carotenoid electronic spectra.^{37,58} The vibronic spacings of $\sim 1200\text{ cm}^{-1}$ thus should be viewed as "hybrids" of the C-C and C=C modes observed in higher resolution polyene spectra. The consistency of the vibronic intervals indicated in Tables 1 and 2 provides additional confidence in the validity of the Gaussian fits and of the electronic origins derived for the $S_1 \rightarrow S_0$ transitions. It is important to point out that the electronic origins ((0-0) bands) for the symmetry-forbidden $S_1 \rightarrow S_0$ transitions should be weak (or nonexistent) with the vibrational progressions built on nontotally symmetric (b_u) promoting modes due to Herzberg-Teller vibronic coupling.⁵⁹ For symmetric polyenes under conditions (e.g., in supersonic jets or in mixed-crystal environments) that retain their centers of inversion, $S_1 \leftrightarrow S_0$ (0-0)'s are missing with the electronic transitions being built on b_u false origins.⁶⁰ However, the most prominent b_u promoting modes are in-plane bending modes with very low frequency, $< 100\text{ cm}^{-1}$ in long polyenes. The distinction between (0-0) bands and b_u false origins thus will not be detectable in the relatively low-resolution spectra (vibronic bandwidths $> 500\text{ cm}^{-1}$) presented here. Furthermore, solvent perturbations and asymmetric substitutions result in (0-0) intensities that are comparable to those of false origins.^{60,61} The electronic origins of $S_1 \leftrightarrow S_0$ spectra in solution thus should be viewed as a complicated superposition of spectra of distorted and undistorted molecules, all of which contribute to the (0-0) line shapes in typical condensed phase spectra.

The present work plus previously published data on longer diapocarotenes provides a systematic view of the $S_0 \rightarrow S_2$ and $S_1 \rightarrow S_0$ transition energies ((0-0) bands) in a common solvent (room temperature *n*-hexane) as a function of conjugation length ($N = 7-11$). These data are summarized in Figure 6. Linear least-squares fits of transition energies versus $1/N$ ($E = A + B/N$) also are indicated (Figure 6) and give $A = 14\,000 \pm 360\text{ cm}^{-1}$ and $B = 66\,000 \pm 3000\text{ cm}^{-1}$ for $S_0 \rightarrow S_2$ and $A = 4500 \pm 300\text{ cm}^{-1}$ and $B = 96\,700 \pm 2600\text{ cm}^{-1}$ for the $S_1 \rightarrow S_0$ transitions. These fits clearly will be useful in extrapolating transition energies to longer diapocarotenoids such as spirilloxanthin ($N =$

studied here, this is approximated by the reciprocal of the measured S_1 lifetime ($\tau_{S_1} = 1/(k_{ic} + k_r) \approx 1/k_{ic}$). ΔE is the $S_1 - S_0$ ($2^1A_g - 1^1A_g$) energy difference, C is a vibronic coupling matrix element, ω_M is the energy of the accepting vibrational mode, and γ can be related to the relative displacement, Δ_M , of the potential surfaces of the S_1 and S_0 electronic states. d is the number of degenerate accepting modes. The dynamics and the fluorescence spectra were measured at room temperature in *n*-hexane solutions, and the rate constants plotted against the values of S_1 energies obtained from the fluorescence spectra. The data points may be fit by the exponential function given in eq 4. Figure 7 shows the fit obtained when the three parameters $m_1, iclo-$ 9.479 0 0 9.479 111.73 346.119 Tm (,ic63.569479 0 0 177(m3dTj 9.46eInic)]TJ -6.1.28 3.mics2i(j /68.4811 1 mfunc1 1 ois)m

energy of spirilloxanthin, the longest naturally occurring di-apocarotenoid. This is still higher than the lowest S_1 state energy for BChl *a* in a light harvesting complex. Thus, from an energetics standpoint, transfer from the S_1 state of any carotenoid to BChl is favorable. However, the exponential increase in the rate constant for radiationless decay of the S_1 states of carotenoids with decreasing energy gap (eq 4) between S_1 and S_0 may reduce the probability that S_1 is the sole energy donor state for the longer conjugated molecules. Transfer from the S_2 (1^1B_u) state to BChl has also been shown to be important in LH2 complexes.^{73,74} It is interesting to note that the best fit line for the diapocarotenoids (Figure 6) predicts $\lambda(0-0) = 524$ nm for the $S_0 \rightarrow S_2$ transition of spirilloxanthin in *n*-pentane. This compares remarkably well with $\lambda(\text{exp}) = 526$ nm,⁷⁵ further supporting the validity of our fits.

Conclusions

These studies on C_{30} carotenoids provide a definitive set of data for the S_1 and S_2 state energies and S_1 state dynamics of open-chain carotenoids. Taken together with data previously published, the energies of the states were found to be well described by the simple linear relationship summarized in Figure 6. The dynamics of the S_1 states of the molecules and the trends in the fluorescence spectral profiles and yields are consistent with an exponential decrease in the nonradiative rate constant for decay from the S_1 state rationalized by the energy gap law for radiationless transitions given by eq 4. The data provide a firm foundation from which the optical spectroscopy and excited state dynamics of structurally more complex carotenoids and xanthophylls may be better understood.

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