Table IV: Experimental and Calculated Values of the $g$ Tensor of Cu(II)-Doped Single Crystals of $\alpha$-Glycylglycine at 4.2 K

<table>
<thead>
<tr>
<th></th>
<th>calculated</th>
<th>experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{xx}$</td>
<td>2.040</td>
<td>2.033*</td>
</tr>
<tr>
<td>$g_{yy}$</td>
<td>2.083</td>
<td>2.085*</td>
</tr>
<tr>
<td>$g_{zz}$</td>
<td>2.236</td>
<td>2.236*</td>
</tr>
</tbody>
</table>

*This work. *Fujimoto et al.12

were assumed. To evaluate the energy matrix the AOM orbital parameters given in refs 43 and 44 were used. The total energy matrix consisting of the ligand field and the spin-orbit coupling (there is no electron–electron repulsion to consider in the d1 and d3 cases) was diagonalized to yield eigenvalues and eigenvectors. The spin–orbit interaction matrix is given in Table I of ref 43.

**Electronic Relaxation in Long Polyenes**

Sarah A. Cosgrove, Melissa A. Guite, Timothy B. Burnell, and Ronald L. Christensen*

Department of Chemistry, Bowdoin College, Brunswick, Maine 04011 (Received: April 26, 1990)

A series of carotenoids with from 7 to 11 conjugated double bonds have been synthesized and purified by using HPLC techniques. Absorption, fluorescence, and fluorescence excitation spectra have been obtained in 77 K glasses. The shorter members of this series exhibit the Stokes-shifted, $S_1 \rightarrow S_0$ emissions seen in previous studies of model polyenes. For carotenoids with more than eight conjugated double bonds, however, the fluorescence is dominated by "anti-Kasha", $S_2 \rightarrow S_0$ fluorescence. These findings in part can be attributed to a larger $S_2-S_0$ energy difference and the resultant decrease in $S_1 \rightarrow S_0$ radiationless decay rates in longer polyenes. The precipitous crossover from $S_1 \rightarrow S_0$ to $S_2 \rightarrow S_0$ emission, however, cannot be fully accounted for by the energy gap law, which predicts only modest changes in radiative and nonradiative decay rates with increasing polyene length. The lack of $S_1$ fluorescence prohibits the direct observation of the $S_1$ state in the longer carotenoids. Nevertheless, trends noted in the shorter polyenes indicate a 5500–6500-cm$^{-1}$ $S_2-S_1$ energy difference for polyenes such as $\beta$-carotene (11 conjugated double bonds). The implications of large $S_2-S_1$ energy gaps for the spectroscopy and photochemistry of $\beta$-carotene and other long polyenes also are discussed.

**Introduction**

The electronic spectroscopy and excited-state dynamics of linear polyenes continue to be topics of considerable interest. Recent experiments range from time-resolved studies of cis–trans isomerization in substituted butadienes,1 to transient Raman spectroscopy of carotenoids bound to membranes of photosynthetic bacteria,2 to investigations of the electronic properties of polyacetylenes.3 These studies encompass a wide range of experimental techniques and theoretical models, and it is not surprising that the relationships between polyenes of different conjugated lengths largely remain unexplored. A common understanding of linearly conjugated systems remains a goal of active interest. This investigation contributes toward this effort by establishing links between the electronic properties of short, model polyenes and the carotenoids, which play important roles in photobiology.

A brief review of previous work on polyenes establishes several distinct areas of interest. Spectroscopic and kinetic investigations of short diphenylpolyenes (from stilbene to diphenyl-octatetraene)4–11 have exploited their commercial availability, stability, high fluorescence yields and relative ease of placement in supersonic jets. Short, unsubstituted polyenes, on the other hand, are not as stable and most notably are nonfluorescent. This has severely hampered their study, and considerably more attention has been focused on unsubstituted or methyl-substituted polyenes of intermediate length.12–23 These compounds combine at least

---

modest fluorescence yields with the ability to be placed in low-
temperature mixed crystals as well as supersonic jets. The
spectroscopy of these model systems has established (for polycenes
with four or more conjugated bonds) the presence of a 2A1g (S1)
state between the ground state (1A1g (S0)) and lowest one-
photon-allowed excited state (1B2g (S2)). Absorption into S1 typ-
ically is followed by a Stokes-shifted emission from S2, with
fluorescence yields being sensitive to molecular structure. Spectra
of intermediate length systems also indicate an increase in the
S2-S1 energy difference with increasing conjugated length.\(^{14,16,21,23}\)

This raises some interesting questions on the energy and properties
of the 2A1g state in long conjugated systems such as \(\beta\)-carotene
and polyacetylene.

Another focus of experimental effort has been on the excited
states of carotenoids. This has been motivated in large part by
the important roles played by carotenoids in photosynthesis both
as antennae pigments\(^{36-39}\) and as photoprotectors.\(^{21}\) The
relationships between these important functions and the properties
of carotenoid excited states remain to be worked out. \(\beta\)-Carotene
(11 conjugated double bonds) has been a particularly popular
subject of investigation. Its strong, broad absorption provides a
good target for laser excitation, and fluorescence measure-
ments\(^{28-34}\) transient absorption experiments,\(^{35,36}\) and 3Raman\(^{37-39}\)
and resonance Raman\(^{40,41}\) spectroscopy have provided many
important details of excited-state structure and dynamics.

Earlier reports of \(\beta\)-carotene’s fluorescence\(^{28}\) have been con-
ﬁrmed by several groups.\(^{29-34}\) However, the fluorescence origin
is not shifted from the origin of the strongly allowed 1A2g (S1)
absorption, indicating that emissions in \(\beta\)-carotene and related
molecules originate from the S2 state. The absence of detectable
S1 \(\rightarrow\) S2 emissions leaves the location of the 2A1g state open to
question. Previous investigations of resonance Raman proﬁles
in \(\beta\)-carotene led to the suggestion\(^{37,38}\) that the 2A1g state lies \(-3500
\text{cm}^{-1}\) below 1B2g and this has been widely cited in discussions
of the antennae function of carotenoids in photosynthesis.\(^{26}\) (The
3500-\text{cm}^{-1} energy gap puts the carotene S1 level well above the
lowest singlet state of chlorophyll.) Recent picosecond transient


![Figure 1. Molecules studied.](Image)
Experimental Section

Synthesis and Purification. 1,3,5,7,9,11,13-Tetradecaheptaene was synthesized as described previously. Reaction mixtures initially were purified by chromatography on Woelm neutral alumina (activity grade II) to separate the heptaene isomers from reactants and shorter polyene impurities. all-trans-Tetradecaheptaene was obtained by reversed-phase HPLC using an Altex Ultrasphere-ODS column and a mobile phase of methanol/water (4/1 v/v). Samples for spectroscopy were collected in the absence of the UV light normally used for detection and extracted into n-pentane. The n-pentane solution was evaporated in a stream of nitrogen, and the residue redissolved in EPA (ether/isopentane/ethanol, 5/5/2 v/v/v). The procedure by which an HPLC peak was collected and converted into a 77 K glass sample was carried out as rapidly as possible under subdued lights to minimize interferences from heptaene degradation products. Sample purities were monitored by reinjection of samples after spectroscopic measurements. Tetradecaheptaene proved the most fragile of the molecules given in Figure 1, and certain of our sample handling procedures (e.g., collection of HPLC samples in the dark and the rapid conversion into low-temperature glasses) were not required to obtain reproducible spectra of the other compounds. 2,4,6,8,10,12,14-Hexadecaheptaene was synthesized from deca-pentenal and crotyltriphenylphosphonium bromide (Instant Ylid, Fluka). The all-trans isomer was obtained by reversed-phase HPLC using an Altex Ultrasphere-ODS column and a mobile phase of methanol/water (95/5 v/v). This fraction was extracted into n-hexane for storage at 20 °C. Dilute samples retained their isomeric purity for several days. However, these procedures did not completely eliminate the presence of less conjugated polyene impurities which could be detected by their low-temperature fluorescence under certain excitation conditions.

β-Apo-12'-carotenol was synthesized by NaBH₄ reduction of β-apo-12'-carotenal, which was a gift from Dr. Percy Manchand of Hoffman-Laroche. After initial recrystallization from ether the alcohol was purified by reversed-phase HPLC (Altex, ODS column) using a mobile phase of methanol/water (95/5 v/v). Due to the low purity (10–20%) of the starting aldehyde, the reaction products contained many visible absorbing components, but the HPLC procedures allowed the isolation of a fraction that could be identified from room-temperature absorption measurements (e.g., wavelength of absorption and the lack of cis peaks) as the all-trans isomer.

β-Apo-8'-carotenol was synthesized by NaBH₄ reduction of β-apo-8'-carotenal (Fluka). Crystallization from ether followed by HPLC (Altex-ODS, 95% methanol/water) allowed the isolation of the predominant all-trans isomer.

β-Apo-6'-carotenol was synthesized by LiAlH₄ reduction of methyl β-apo-6'-carotenoate which was obtained by a Wittig reaction between β-apo-8'-carotenol and trimethyl phosphonoacetate. Trimethyl phosphonoacetate (Aldrich, 110 mg) and NaH (15 mg) were dissolved in 20 mL of anhydrous DMF and stirred under argon gas in the presence of molecular sieves. After 30 min, 250 mg of β-apo-8'-carotenol (Fluka) in 2 mL of DMF was added, and the solution stirred for 15 h at room temperature. The reaction mixture was then diluted with water, and the organic components were extracted into ether, which was then washed and evaporated to yield the ester. Reduction of the ester was carried out at 30 °C in a closed argon syringe. After 90 min the reaction was quenched by the addition of ethyl acetate. Workup of the organic fractions gave crude β-apo-6'-carotenol in 90% overall yield. The all-trans isomer was isolated by reversed-phase HPLC (Altex, ODS column) using a mobile phase of 100% methanol.

Isoxazaxanthin was obtained by LiAlH₄ reduction of canthaxanthin (Fluka). After recrystallization from methanol the crude product was purified on an ODS column using 100% methanol as the mobile phase. Due to the high purity of the starting materials, the chromatograms showed a dominant component that could be identified as all-trans-isoxazaxanthin.

Spectral Measurements. Absorption spectra of room-temperature solutions and 77 K glasses were obtained on a Shimadzu

Figure 2. Fluorescence and fluorescence excitation spectra of all-trans-2,4,6,8,10,12,14-hexadecaheptaene in 77 K n-pentane. The fluorescence spectrum was obtained by exciting at 414 nm. The fluorescence excitation spectrum was obtained by monitoring the emission intensity at 558 nm.

UV240 spectrophotometer interfaced to a microcomputer. Low-temperature absorption spectra were obtained by using a square quartz cuvette and a flat window, suprasil liquid nitrogen dewar. Fluorescence and fluorescence excitation spectra were obtained by using a cylindrical suprasil dewar and cell on a SPEX Model 212 spectrophotometer equipped with a SPEX DMIB data station. All spectra were corrected for the wavelength dependencies of optical components and then transferred to a mainframe computer for subsequent analysis and display. In some cases spectra were subjected to mild smoothing to reduce the noise inherent in these weakly emitting systems. These procedures only minimally distorted the rather broad spectral details of these spectra.

The fluorescence quantum yield of isoxazaxanthin was obtained by comparing its fluorescence intensity (integrated with respect to frequency) with that of fluorescein (Φf = 0.90 in 0.1 N sodium hydroxide). Although isoxazaxanthin and fluorescein have comparable absorption and emission spectra, the large disparity between their emission yields required the use of dilute fluorescein solutions to obtain emission spectra of isoxazaxanthin and the standard under the same electronic and optical conditions.

Results

Spectra of the two simple heptaenes provide the link between the present study and the previous work on shorter model polyenes. The 77 K fluorescence and fluorescence excitation spectra of hexadecaheptaene are given in Figure 2. The corrected excitation spectrum is in excellent agreement with the absorption spectrum, and the fluorescence spectrum is independent of excitation wavelength. These observations confirm that the HPLC procedures have resulted in a single-component sample. The hexadecaheptaene spectra are very similar to 77 K spectra obtained for shorter hexaenes, pentaenes, and tetraenes, showing the classic Stokes shift between the electronic origins of absorption (λ = 414 nm) and emission (λ = 514 nm). The 4700-cm⁻¹ gap between the origins in Figure 2 thus corresponds to the energy difference between the 1B1g and 2A1g excited states.

The 77 K absorption and fluorescence spectra of tetradecaheptaene are given in Figure 3. Comparison of the corrected excitation spectrum with the absorption spectrum and theob
Electronic Relaxation in Long Polyenes

Figure 3. Absorption and fluorescence spectra of all-trans-1,3,5,7,9,11,13-tetradecaheptaene in 77 K EPA. The absorption spectrum was obtained with a 2-nm bandpass. The fluorescence spectrum was excited by using the 405.4-nm line from a Hg lamp and detected with a 1-nm bandpass through a Schott KV-418 filter (see footnote 46). Observation that the fluorescence spectrum is not strictly independent of the excitation wavelength indicate the presence of shorter polyenes, even in these highly purified samples. In spite of these interferences, the heptaene fluorescence can be isolated by excitation into the absorption origin at 402 nm. This results in the well-resolved fluorescence spectrum given in Figure 3. Excitation into higher energy vibronic levels (e.g., the strong vibronic band at 383 nm) gives emissions with decreased resolution and increased resolution into the absorption origin at 402 nm. This results in the well-resolved fluorescence spectrum given in Figure 3. Excitation into higher energy vibronic levels (e.g., the strong vibronic band at 383 nm) gives emissions with decreased resolution and increased intensity at shorter wavelengths. A series of fluorescence spectra taken over a range of excitation wavelengths establish the emission origin at 408 nm and eliminate the possibility that the shorter-wavelength emissions are due to Raman or resonance Raman scattering. Similarly, a series of excitation spectra taken at different monitoring wavelengths gave spectra that reproduce all the vibronic features of the tetraene absorption.

The 77 K spectra of hexadecaheptaene and tetradeceaheptaene offer some interesting contrasts. Most obvious is the absence of an energy gap between the 1'Ag - 1'Bg absorption and the onset of the fluorescence, suggesting 1'Bg - 1'Ag emission. In addition to the relatively strong fluorescence in the 410-500-nm region, tetradeceaheptaene also shows some weak but reproducible vibronic features at λ ≥ 511 nm. Excitation spectra monitored on these bands also closely correspond with the absorption spectrum. These weak emissions are almost identical to the 2'A, fluorescence observed for hexadecaheptaene. We thus must conclude that in tetradeceaheptaene emission occurs from both 1'Bg (S2) and 2'A (S1) with S2 - S0 emission dominating.

Previous solvent-shift studies on room-temperature absorption and fluorescence spectra confirm the assignment of dual emissions in tetradeceaheptaene. The 402-nm absorption band and the 410-500-nm emission features exhibit comparable shifts in a series of hydrocarbon solvents. The dependence of these shifts on solvent polarizability also is typical of shifts of observed for the strongly allowed 1'Ag - 1'Bg transition in shorter polyenes. In contrast, the longer wavelength emissions at λ ≥ 511 nm show a much weaker dependence on solvent polarizability, comparable to solvent shifts previously observed for forbidden 2'A - 1'A transitions. The solvent studies also help in identifying the 2'A - 1'A (0-0) at 511 nm, since all the vibronic features at shorter wavelengths exhibit the polarizability dependences of allowed transitions. In addition, the 514 nm S0 - S1 electronic origin in hexadecaheptaene (Figure 2) strongly supports our assignment.

The 77 K excitation and emission spectra of β-apo-12'-carotenol are compared with spectra of hexadecaheptaene in Figure 4. Spectra of the carotenol are noticeably broader due to well-understood complications brought about by the cyclohexenylidene ring. Nevertheless, there is sufficient resolution in the carotenol spectra to allow the identification of the electronic origins in both emission and excitation. The spectra of β-apo-12'-carotenol and hexadecaheptaene are then seen to be quite similar with almost identical electronic energies and a clear indication of 2'A, - 1'A, fluorescence. In contrast to β-apo-12'-carotenol, the low-temperature fluorescence from β-apo-8'-carotenol is dominated by 1'B - 1'A, (S2 - S0) emission (see Figure 5). The long wavelength region (600-700 nm) of the fluorescence spectrum is perhaps suggestive of weak 1'S - S0 emission (compare with Figure 3), but the broad, unresolved spectral tail does not allow identification of vibronic features similar to those seen in tetradeceaheptaene. The fluorescence characteristics of β-apo 6'-carotenol (Figure 6) are almost identical with those exhibited by β-apo-8'-carotenol. For both of these molecules the fluorescence excitation spectra are in excellent agreement with the well-resolved 1'A - 1'B absorption, and the fluorescence spectra are independent of the wavelength of excitation. These features plus the good overlap between absorption and emission origins strongly support the assignment of "anti-Kasha", S0 - S0 emission in these systems.

Isozoexanthin also emits from its S2 state (Figure 7). The 77 K absorption and excitation spectra are in excellent agreement,

and second excited singlets. Similar violations of Kasha’s rule subsequently were observed for derivatives of azulene, metalloporphyrins, and several polyenes. All of these molecules are characterized by large \( S_2-S_1 \) energy differences, e.g., 6900 cm\(^{-1}\) in zinc tetrabenzoporphyrin and 7000 cm\(^{-1}\) in acenaphthylene.

The theoretical basis for the \( S_2 \rightarrow S_0 \) emissions in azulene and other cyclic aromatics was provided by Robinson and Frosch and by Englman and Jortner in their general treatments of radiationless transitions in large molecules. These molecules can be described in the “weak coupling limit” in which the displacement between the potential energy surfaces of different electronic states is relatively small. This leads to the well-known energy gap law, which predicts an exponential decrease of the internal conversion rate with increasing energy difference between electronic states. The energy gap law has been used to analyze radiationless decay processes in a wide variety of organic and inorganic molecules and appears to give a good account of the violations of Kasha’s rule cited above.

Long polyenes are, at first glance, well-suited for exhibiting \( S_2 \rightarrow S_0 \) fluorescence. The increase in the \( S_2-S_1 \) energy gap (\( \Delta E \)) with increasing conjugation thus should be accompanied by a decrease in the rate of \( S_2 \rightarrow S_1 \) internal conversion (\( k_{21} \)). For polyenes with seven or eight double bonds \( k_{21} \) apparently becomes small enough to allow the detection of \( S_2 \) fluorescence (recall that the \( S_2 \rightarrow S_0 \) fluorescence quantum yield \( = k_{20}/(k_{20} + k_{21}) \), where \( k_{20} \) is the rate of radiative decay from \( S_2 \)). \( S_2 \) emission also is favored by the relatively large \( k_{20} \) provided by the symmetry-allowed \( S_2 \rightarrow S_1 \) transition. It is interesting to note that \( S_2 \) emission in polyenes makes its appearance at energy differences (\( \Delta E > 5000 \text{ cm}^{-1} \)) comparable to those required to observe \( S_2 \) emissions in aromatic systems. This suggests that many of the parameters contained in the energy gap law (the changes in molecular geometry, the energies and number of C-H “acceptor models”, vibronic coupling parameters, etc.) are similar for polyene and aromatic systems.

While the energy gap law gives a qualitative explanation for the appearance of \( S_2 \) emission in longer polyenes, the crossover from the Stokes-shifted \( S_2 \) emissions of shorter polyenes to the dominant \( S_2 \) emissions of longer polyenes is quite abrupt given the rather gradual increase in the \( S_2-S_1 \) energy gap. This can be seen in the comparison of the fluorescence spectra of \( \beta \)-apo-12'-carotenol (Figure 4) and \( \beta \)-apo-8'-carotenol (Figure 5). The \( S_2-S_1 \) energy difference (\( \Delta E \)) in the shorter carotenoid is similar to that of hexadecapentaene (\( \sim 4700 \text{ cm}^{-1} \)). On the basis of our work on shorter unsubstituted and \( \alpha \)-w-substituted polyenes, we estimate \( \Delta E = 5500 \pm 200 \text{ cm}^{-1} \) for \( \beta \)-apo-8'-carotenol in a 77 K EPA glass. Substituting these \( \Delta E \)'s into the weak-coupling limit equation given by Englman and Jortner and assuming that the parameters used for azulene and several aromatic systems also are appropriate for the polyenes) gives an \( S_2 \rightarrow S_1 \) internal conversion rate that is not more than a factor of 2 in changing from \( \beta \)-apo-12'-carotenol to \( \beta \)-apo-8'-carotenol.

A more detailed analysis of the radiative and nonradiative processes connecting \( S_2, S_1, \) and \( S_0 \) leads to the following equation:

\[
\Phi_1/\Phi_2 = (k_1/k_2)(k_{10}/k_{20})
\]

where \( \Phi_1 \) and \( \Phi_2 \) are the fluorescence yields from \( S_1 \) and \( S_2 \), respectively.


(56) Robinson, G. W.; Frosch, R. P. J. Chem. Phys. 1962, 37, 1962; 1963, 38, 1187.


following $S_0 \rightarrow S_1$ absorption, $k_{21}$ is the rate of conversion between $S_2$ and $S_1$, $k_1$ is the rate of depopulation of $S_1$ ($=k_{12}+k_{1r}$), $k_{12}$ is the radiative decay rate from $S_1$, and $k_{1r}$ is the radiative decay rate from $S_0$. This model assumed that $S_1$ is populated only via $S_0$. Also, the extremely low radiative decay rate means that $k_1 \approx k_{1r}$ for the longer polyenes considered in this paper.

Increasing the length of conjugation will change all of the rate constants indicated in eq 1. The lowering of the $S_0$-$S_1$ energy gap will increase $k_{1r}$, again following the energy gap law. In addition the larger $S_0$-$S_2$ gap should reduce $k_1$, due to the decrease in mixing between $S_1$ and $S_2$. This mixing provides oscillator strength to the symmetry-forbidden $2^1A_g \rightarrow 1^1A_g$ transition and should vary inverse quadratically with the $S_0$-$S_1$ energy difference. Finally, $k_{21}$ should show a mild increase with polyene length. This accounts for the general trend from $S_1$ emission in short polyenes to $S_2$ emission in long polyenes. On the other hand, even with the most extreme (but still physically reasonable) choices for energy differences and parameters, we can account for no more than a factor of 5 change in $\Phi_1/\Phi_2$ in changing from $\beta$-apo-12'-carotenol ($\Phi_1/\Phi_2 > 10$) to $\beta$-apo-8'-carotenol ($\Phi_1/\Phi_2 < 10$). We must conclude either that nonradiative processes in polyenes are governed by parameters of different magnitudes (giving a steeper dependence on energy gap) than those that apply to aromatic systems or that there are additional effects (other than those considered in the energy gap model) that control electronic relaxation in polyenes.

Comparison of the fluorescence spectra of hexadecaheptaene (Figure 2B) and $\beta$-carotene (Figure 2C) shows that the fluorescence yield for the former has approached that of substitution also may change the rate constants of eq 1. The two heptaenes have comparable $S_0$-$S_2$ energy differences (4700 cm$^{-1}$ for hexadecaheptaene and 5300 cm$^{-1}$ for tetracosaheptaene) but large differences in the ratio of their $S_1$ to $S_0$ and $S_2$ to $S_0$ emissions. We recently have observed a similar pattern in the gas-phase fluorescence of tetracosaheptaene. The emission spectrum of the unsubstituted octatetraene ($\Delta E = 6600$ cm$^{-1}$) exhibits $S_2 \rightarrow S_1$ and $S_0 \rightarrow S_1$ transitions. The long wavelength that may originate from the heavy substitution in the polyene backbone, an assignment widely cited in discussions of energy transfer between carotenoids and chlorophylls in photosynthesis. The Raman excitation and transient absorption experiments thus seem to lead to the same conclusion, i.e., the $S_0$-$S_2$ energy difference in $\beta$-carotene is $\leq 3500$ cm$^{-1}$.

Placement of the $2^1A_g$ state of $\beta$-carotene in the 17000 - 18 500 cm$^{-1}$ (540 - 590 nm) range, however, is not consistent with energy trends observed in shorter polyenes. The $2^1A_g \rightarrow 1^1A_g$ transitions of shorter polyenes show a smooth progression toward longer wavelengths as a function of polyene length. For example, the $\alpha,\omega$-dimethyl polyenes have $S_1 \rightarrow S_0$ transitions at 565 nm, in good agreement with the 560-nm origin observed for the simple, $\alpha,\omega$-methyl-substituted octadecaheptaene. We therefore conclude that $2^1A_g$ energies observed for model polyenes provide good estimates of $2^1A_g$ energies in carotenoids of the same conjugated length. Extrapolation of data on the shorter model polyenes listed above indicates an $S_1 \rightarrow S_2$ electronic origin of $\sim 700$ nm for isoxanthaxin and $\beta$-carotene, putting the $2^1A_g$ state at a substantially lower energy than suggested by the Raman excitation and transient absorption measurements.

The lack of $S_1 \rightarrow S_0$ emission in longer carotenoids also is not consistent with a small (i.e., $\Delta E \leq 3500$ cm$^{-1}$) $S_0$-$S_1$ energy difference. Examination of room-temperature $n$-hexane solutions of the $\alpha,\omega$-dimethyl series shows the following $\Delta E$: tetracene (4600 cm$^{-1}$), pentaene (5000 cm$^{-1}$), hexaene (5600 cm$^{-1}$), and heptaene (5900 cm$^{-1}$). These energy differences are lower for carotenoids in low-temperature glasses due to stabilization of $1^1B_g$ relative to $2^1A_g$. Thus $\Delta E$ for $\beta$-apo-12'-carotenol in 77 K EPA (Figure 3) falls from 5900 cm$^{-1}$ to 4700 cm$^{-1}$. In any event the energy gap also is not the cause of the $S_0$-$S_1$ electronic origins at 565 nm, in good agreement with the 560-nm origin observed for the simple, $\alpha,\omega$-methyl-substituted octadecaheptaene. We therefore conclude that $2^1A_g$ energies observed for model polyenes provide good estimates of $2^1A_g$ energies in carotenoids of the same conjugated length.

Regardless of the reasons for the large sensitivity of the fluorescence yield ratio to conjugated length, the trends we have observed have important implications for longer polyenes such as $\beta$-carotene. Our experiments indicate that $2^1A_g$ states in polyenes with more than eight or nine double bonds will not be easily detected in standard fluorescence or absorption experiments. Nevertheless, in $\beta$-carotene and related compounds the $2^1A_g$ state lives long enough to be characterized by transient absorption$^{52-54}$ and resonance Raman experiments.$^{54}$ These measurements indicate $\sim 10^{-10}$-s lifetimes, which are in agreement with ground-state depletion studies on these molecules. The $2^1A_g \rightarrow S_0$ fluorescence yield for 77 K isoxanthaxin is comparable to that of $\beta$-carotene.$^{52}$ Our inability to detect $S_0$ emission in isoxanthaxin suggests a $S_1 \rightarrow S_0$ fluorescence yield of $< 10^{-4}$. This combined with the $10^{-11}$-s lifetime indicates an $S_1$ intrinsic lifetime of $> 10^{-9}$ s and a $S_0 \rightarrow S_1$ oscillator strength that is at least $10^{5}$ times smaller than that of the $S_0 \rightarrow S_2$ absorption (10$^{-9}$s intrinsic lifetime).

The lack of $S_1 \rightarrow S_0$ emission and the forbidden (or at least extremely weak) nature of the $S_0 \rightarrow S_1$ absorption means that the $S_1$ state in $\beta$-carotene, isoxanthaxin, and other long polyenes must be detected by other than conventional optical techniques. For example, the Raman excitation profile of $\beta$-carotene in the 18000 cm$^{-1}$, which were identified as vibrionic bands of $2^1A_g$,$^{35,37}$ This leads to an $\sim 3500$-cm$^{-1}$ $S_0$-$S_1$ energy gap and placement of $2^1A_g$ well above the $\sim 15000$-cm$^{-1}$ origin of the lowest energy absorption in chlorophyll, an assignment widely cited in discussions of energy transfer between carotenoids and chlorophylls in photosynthesis.$^{25,26,39}$

emission. Quenching of $S_1$ emission requires a large $\Delta E$.

Consideration of polyene $S_1$ energies and $S_2-S_1$ energy differences thus requires a reevaluation of the Raman excitation and transient absorption experiments. Of particular significance is a more recent investigation of the Raman excitation profile of beta-carotene. Watanabe et al. investigated the preresonance region and found no evidence for the $2A_g$ state. These experiments indicate that this technique (like standard absorption measurements) is not capable of detecting the weak $1A_g \rightarrow 2A_g$ transition on top of the strong $1A_g \rightarrow 1B_g$ absorption tail. The transient absorption measurements appeared to support an even smaller $1B_u-2A_g$ energy difference ($\sim 2400$ cm$^{-1}$). However, such experiments are difficult to interpret. Connecting the $S_1 \rightarrow S_n$ and $S_0 \rightarrow S_m$ absorption spectra is fraught with the obvious difficulty in identifying a common final state ($n = m$), especially for a large molecule such as beta-carotene where there are many electronic states in the neighborhood of $S_n$ and $S_m$. Theory is of little or no help in assigning transitions for a polyene of this size. The problems in assigning excited state spectra are best illustrated by the transient absorption spectrum of diphenyloctatetraene. Initial assignments of nanosecond time-scale $S_1 \rightarrow S_n$ spectral features were based on an apparently reasonable theoretical model. However, recent picosecond $S_1 \rightarrow S_n$ measurements lead to a revision in the original assignments and cast doubt on the model's ability to distinguish between transitions of comparable energy and oscillator strength. We therefore suggest that the 555-nm transient absorption in all-trans-beta-carotene does not originate in the state responsible for the 275-nm absorption. The results of the Raman excitation experiments and the interpretation of the transient absorption experiments thus are very much open to question.

Replacing the previous picture of a relatively narrow $S_0-S_1$ energy gap in long carotenoids opens up some interesting possibilities for energy transfer between molecules such as beta-carotene and various chlorophylls. If our extrapolation of the $2A_g$ state to 650–700 nm is correct, then beta-carotene and chlorophyll would have $S_1$ states of comparable energies. On the other hand, the $S_1$ state of beta-carotene and longer carotenoids (e.g., spirilloxanthin, with 13 conjugated double bonds) may indeed be lower than the lowest energy singlet in certain chlorophylls. One then might need to invoke higher energy electronic states (e.g., $S_2$) in the energy transfer mechanism. It also should be noted that our rather limited experimental and theoretical understanding of the electronic levels in long polyenes does not preclude the existence of additional $A_g$ states lying below $1B_u$. This complication might explain the abrupt changeover to $1B_u$ emission in the longer polyenes and account for the transient absorption experiments discussed above while also providing additional mechanisms for energy transfer between carotenoids and chlorophylls.

Refinement of these speculations awaits experiments that more accurately locate low-lying $1A_g$ states in long polyenes. Particularly promising in this regard would be the direct measurement of the $S_1-S_0$ energy gap in $S_1 \rightarrow S_2$ absorption experiments. Bachilo and Bondarev recently reported transient absorption in the near IR (1000–1650 nm) for excited diphenyloctatetraene and assigned this to a high-energy vibronic band of the $2A_g \rightarrow 1B_u$ transition. The energy of this transition and its sensitivity to solvent changes appear to conform to $S_0-S_2$ energy differences obtained from the Stokes shift between the absorption and emission in room-temperature solutions. However, the low $\Delta E$ ($\sim 3000$ cm$^{-1}$) for diphenyloctatetraene prevented the direct observation of the $S_0 \rightarrow S_2$ (0-0) and other vibronic bands. For polyenes of conjugated lengths comparable to beta-carotene we estimate a considerably larger $\Delta E$, $\sim 5500-6500$ cm$^{-1}$. The electronic origin of $2A_g \rightarrow 1B_u$ thus should occur at 1500–1800 nm with vibronic structure extending toward the visible. Extension of the previous $S_1 \rightarrow S_n$ measurements on beta-carotene into the near IR thus should accurately locate the $2A_g$ state in this important molecule. Application of this technique to longer and shorter polyenes also would provide a much better indication of the energy of $2A_g$ in the long polyene limit.

**Acknowledgment** is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, a Penta Corporation grant of the Research Corporation, and a Du Pont Fund grant to Bowdoin College for support of this research. We thank Dr. Percy Manchand of Hoffman-LaRoche for gifts of carotenoids and advice on synthetic routes. We thank D. Scott Smith for preliminary studies and T. Gilbro, H. Petek, and H. Okamoto for helpful discussions. R.L.C. thanks Professor Keitaro Yoshihara and the Institute for Molecular Science for their hospitality during the completion of this paper. A grant from the Japan Ministry of Education, Science, and Culture also is gratefully acknowledged.

---