S_1 and S_2 States of Apo- and Diapocarotenes

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A series of apocarotenes with 5 to 11 conjugated double bonds were synthesized and all-trans isomers were isolated using HPLC techniques. Absorption, fluorescence, and fluorescence excitation spectra were obtained in 77 K glasses. As previously noted for other polyenes and carotenoids, fluorescence spectra of the apocarotenes exhibit a systematic crossover from $S_1(2^1A_g)$
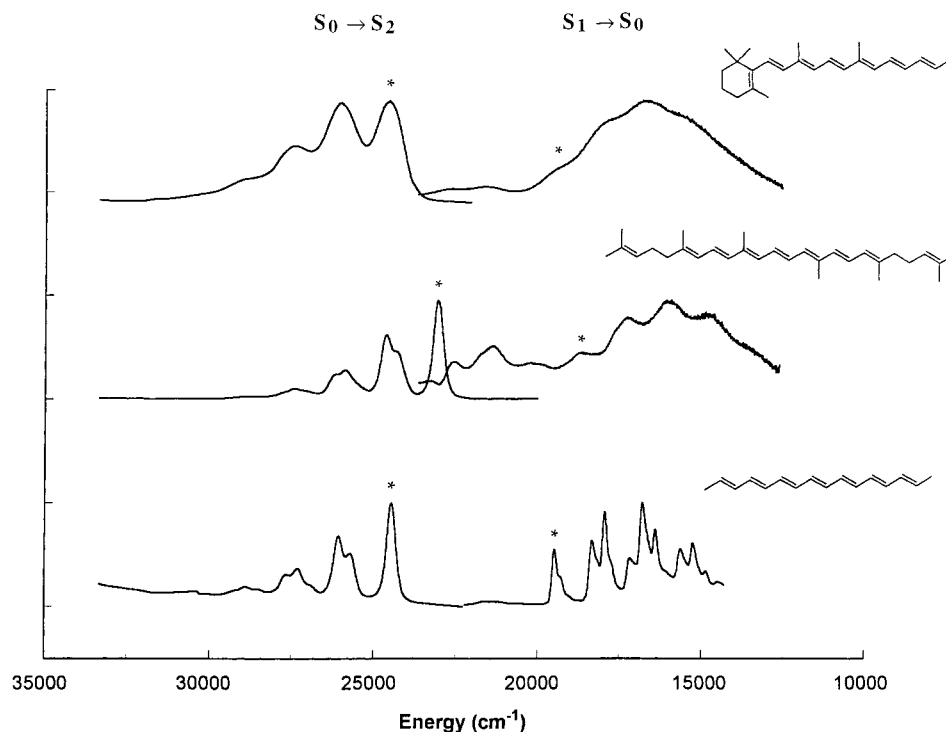
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Figure 2. Comparison of absorption and fluorescence spectra of *all-trans*-heptaenes: hexadecaheptaene (bottom), 7,8,7′,8′-tetrahydro-4,4′-diapocarotene (middle), and β-apo-12′-carotene (top). Spectra were obtained at 77 K in an EPA glass (ether/isopentane/ethanol, 5/5/2, v/v/v). Electronic origins are indicated by asterisks.

(ether/isopentane/ethanol, 5/5/2, v/v/v) for low-temperature optical studies. Samples were reinjected into the HPLC before and after exposure to light to ensure that the spectra obtained were those of the all-trans isomers.

Absorption spectra of room temperature solutions and 77 K glasses were acquired on a Shimadzu UV240 spectrophotometer interfaced to a microcomputer. Low-temperature absorption spectra were obtained using a square quartz cuvette and a liquid nitrogen cryostat with flat Suprasil windows. Fluorescence and fluorescence excitation spectra were obtained using a cylindrical Suprasil cryostat and cell on a SPEX Model 212 spectrofluorimeter. All spectra were corrected for the wavelength dependencies of optical components. In converting spectra from wavelength to frequency (cm^{-1}) scales, the data also were corrected for the differences between band-passes at constant wavelength vs constant wavenumber resolution, i.e., $I(\tilde{\nu})/d\tilde{\nu}$) $\lambda^2 I(\lambda)/d\lambda$. In some cases spectra also were subjected to mild smoothing[34,35] to reduce the noise inherent in these weakly emitting systems. These procedures only minimally distorted the rather broad spectral details of these spectra. The positions of vibronic bands in emission and absorption were determined by fitting spectra to sums of a small number (3- 7) of Gaussian-shaped bands using Square Tools, a utility operating within SpectraCalc (Galactic Software). Peak positions of prominent bands and shoulders were averages obtained from a range of fits using different numbers of Gaussians and different approximations for baselines.

Results and Discussion

Major differences between the electronic spectroscopy of simple polyenes and carotenoids are illustrated in Figure 2 which compares the low-temperature absorption and fluorescence spectra of three *all-trans*-heptaenes (Figure 1): hexadecaheptaene, 7,8,7′,8′-tetrahydro-4,4′-diapocarotene, and β-apo-12′-apocarotene. Spectra of hexadecaheptaene are well resolved with

well-defined electronic origins, allowing the accurate measurement of S$_1$(2^{1}A$_g$) and S$_2$(1^{1}B$_u$) electronic energies. The addition of methyl substituents and alkyl end groups to form the isoprenoid diapocarotene broadens the vibronic bands and shifts the electronic transitions to lower energy. The S$_0$(1^{1}A$_g$) $\rightarrow$ S$_2$-(1^{1}B$_u$) transition energy is considerably more sensitive to alkyl substitution than the S$_0$(1^{1}A$_g$) $\rightarrow$ S$_1$(2^{1}A$_g$) transition. This is a common feature of polyene/carotenoid spectroscopy and mimics the relative sensitivities of the 2^{1}A$_g$ and 1^{1}B$_u$ energies to solvent perturbations.[1] Introduction of the terminal β-ionylidene ring in the apocarotene further broadens the spectra and shifts the S$_0$(1^{1}A$_g$) $\rightarrow$ S$_2$(1^{1}B$_u$) transition to substantially higher energy relative to the absorption spectrum of the corresponding diapo compound. The apocarotene S$_1$(1^{1}A$_g$) $\rightarrow$ S$_0$(2^{1}A$_g$) bands experience a significantly smaller blue shift relative to the diapo analogue.

The blue shifts and the loss of resolution in spectra of the apocarotene can be traced to the repulsions between methyl groups on the β-ionylidene ring and the hydrogen atom at the end of the C$_{7-8}$ double bond (Figure 1) in the polyene chain.[36,37] 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. 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.[36,37] As a result, solutions of apocarotenes, β-carotene, etc. typically contain a distribution of conformers with a range of effective conjugation lengths and transition energies. This simple model also explains the significant blue shift in the apocarotene relative to the diapocarotene. The open-chain diapocarotene enjoys the full effect of conjugation, while the broader apocarotene spectra are due to a superposition of spectra of species with conjugation lengths that average between six (90° rotation about C$_{6-7}$) and seven (no rotation) conjugated bonds.

modest increase in resolution partially offsets lower fluorescence yields, allowing the detection and identification of $S_1 \rightarrow S_0$ vibronic bands in longer apocarotenes and other carotenoids.

The vibronic structure observed in the low-temperature spectra of the apocarotene series proves to be sufficient to accurately locate $S_0 \rightarrow S_2$ and $S_1 \rightarrow S_0$ vibronic bands, including the electronic origins. The spectra presented in Figure 3 were fit to a superposition of Gaussian-shaped bands using standard least-squares procedures. The number of Gaussians (and their widths and amplitudes) used for relatively unresolved spectra (e.g., N) 5) was compared with those obtained from similar fits of more resolved spectra of apocarotenes (e.g., N) 8). Spectra such as those of hexadecaheptaene (Figure 2) also were used as templates to ensure that the least-squares fits on the apocarotenes were consistent with the vibronic patterns observed for simple polyenes. Peak positions obtained by varying the number of Gaussians (3- 8) and the nature of the baselines were averaged to estimate the positions of the dominant vibronic bands and shoulders of the spectra presented in Figure 3. The results of these fits (and their estimated errors) are presented in Table 1. We also have detected in β-apo-6$'$-carotene (N) 10) a weak, partially resolved $S_1 \rightarrow S_0$ emission on the tail of the dominant $S_2 \rightarrow S_0$ transition (Figure 3). However, the spectrum is not of sufficient quality to allow accurate deconvolution into its vibronic components.

Figure 4 compares the apocarotene emission spectra on a scale on which the emission spectra have been shifted to force the $S_1 \rightarrow S_0$ (0- 0)'s (Table 1) to share a common zero of energy. The remarkable similarities between these spectra provide strong confirmation that our deconvolution of vibronic components gives an accurate account of the energies of electronic origins as well as other prominent vibronic features. (It should be noted that the consequences of conformational disorder make it difficult to unambiguously assign the vibronic bands in the broad spectra of axerophtene (N) 5). In this instance, we also have been guided by the vibronic energies of longer members of the apocarotene series; see Figure 5.) Comparisons with the $S_1 \rightarrow S_0$ spectrum of the simple heptaene provide further support forn 53accur.m -0.2accur5am -0 0 Tn5am -0in5am -0 m -0le (N-origre

origins.[28,46,47] Furthermore, these bands undergo inhomogeneous solvent shifts due to the distribution of local solvent environments.[46] As a result, the electronic origins of $S_0 \leftrightarrow S_1$ spectra of polyenes and carotenoids 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)" band shapes in Figures 3 and 4.

Figure 5 summarizes the $S_0 \rightarrow S_2$ (0- 0) energies and the energies of the dominant vibronic components of the $S_1 \rightarrow S_0$ transitions as a function of the number of conjugated double bonds. The $S_1 \rightarrow S_0$ vibronic energies vary almost linearly with N, and we have included best fit lines as a guide for summarizing the data. (Theory suggests that E) A - B/N for large N, but plots of the apocarotene energies versus $1/N$ show large curvatures over the limited range of conjugation (N) 5- 9 for the $S_1 \rightarrow S_0$ vibronic bands) for which we have reliable data.) In spite of the inherent limitations of extrapolations, such plots naturally encourage speculation on the S_1 energies of slightly longer apocarotenes. Given their connections with the naturally occurring carotenoids employed in photosynthetic antenna, namely spheroidene and β-carotene (Figure 1), it is particularly interesting to extrapolate to the S_1 energies of the apocarotenes with N) 10 and N) 11. To this end, we have superimposed on the graph of apocarotene electronic energies the S_1 energies of chlorophylls found in the antenna of plants (Chl$a$ (Q_y)) and bacteria (BChl$a$ (Q_y)). Regardless of the details of the extrapolation, Figure 5 indicates that the 2^1A_g state of spheroidene must lie well above the S_1

approximately additive. S

1000 cm^{-1} change in ΔE which clearly is not the case for these molecules. This analysis and the comparisons provided by Figure 6 reinforce the notion that, in spite of the great usefulness of eq 1 in rationalizing fluorescence yields and lifetimes,[51,52] the dynamics of carotenoid S_1 states depend on the details of molecular structure as well as the S_1- S_0 energy gap. Energy gap law extrapolations thus will be most accurate when comparing molecules with similar structures and conjugation lengths.

Conclusions

The apocarotene series bridges the gap between molecules such as β-carotene and open-chain carotenoids such as lycopene and spheroidene. Unlike β-carotene and its analogues, the low-temperature absorption and emission spectra of the apocarotenes are sufficiently resolved to allow the identification and accurate measurement of $S_0 \leftrightarrow S_2$ and $S_1 \rightarrow S_0$ vibronic bands, including the energies of electronic origins. The apocarotene spectra presented in this paper provide a considerably more accurate catalog of 2^1A_g and 1^1B_u energies as a function of conjugation length than those obtained from previous studies of synthetic carotenoids and spheroidenes.

Comparisons with comparable diapocarotenes provide quantitative measures of the increases in $S_0 \rightarrow S_2$ and $S_1 \rightarrow S_0$ transition energies when terminal double bonds are embedded in cyclohexenyl rings. Differences in transition energies decrease with increasing conjugation, and $S_1 \rightarrow S_0$ transitions are significantly less sensitive to structural differences than $S_0 \rightarrow S_2$ transitions. The presence or absence of terminal rings appears to have a much more significant impact on the dynamics of carotenoid excited states. These results echo earlier observations on model tetraenes and heptaenes which indicate the important role of terminal methyl substituents in controlling $S_2 \rightarrow S_0$ and $S_1 \rightarrow S_0$ fluorescence yields.[20,21] In addition, carotenoids containing keto and allenic or acetylenic groups show significant variations in $S_1 \rightarrow S_0$ lifetimes that are not accompanied by corresponding changes in the $S_1 \rightarrow S_0$ transition energies.[59,60] These results suggest that some caution should be used in applying "universal" energy gap law expressions to a wide range of polyenes and carotenoids, particularly in estimating S_1 energies from the S_1 lifetimes of species with significant variations in molecular structure.

Given their relative ease of synthesis and the tabulations of S_1 and S_2 energies provided by this study, the apocarotenes should be viewed as attractive targets for calibrating new spectroscopic techniques before extending these approaches to longer, nonfluorescent polyenes and carotenoids. Due to its photobiological importance and the commercial availability of high-purity samples, β-carotene has been the almost exclusive target for the initial application of a wide variety of spectroscopic techniques to elucidate energies and properties of "long carotenoid" 2^1A_g states. Recent publications claiming the detection of the 2^1A_g state in β-carotene include the use of reflectance spectroscopy in zeolite[61] and 4.2 K single crystals[55] and the electric-field-induced change in the absorption (electroabsorption) in polystyrene matrices.[62] Another intriguing result is the observation of a weak absorption background in the inverse Raman (Raman loss) spectrum of a related carotenoid, canthaxanthin.[63] By far the most promising of recent experiments on longer carotenoids are the reports of the detection of 2^1A_g states in the resonance Raman excitation spectra of 4.2 K single crystals of β-carotene[55] and in solid *all-trans*-spheroidene.[64] The potential of resonance Raman excitation techniques to detect $S_0 \rightarrow S_1$ absorptions in long carotenoids provides a tantalizing alternative to the detection of extremely weak fluorescence signals and/or the use of energy gap and other extrapolation techniques. However, the use of resonance Raman excitation techniques to uncover 2^1A_g states in carotenoids has a troubled past.[21,65,66] Resonance Raman and other techniques thus would benefit from systematic calibration on shorter polyenes and carotenoids whose 2^1A_g energies can be unambiguously established by detection of vibronically resolved, $S_1 \rightarrow S_0$ fluorescence. This would allow the straightforward evaluation of the ability of these alternate techniques to detect $1^1A_g \rightarrow 2^1A_g$ transitions in spheroidene, β-carotene and other carotenoids of biological interest.

References and Notes

(1) Hudson, B. S.; Kohler, B. E.; Schulten, K. In *Excited States*; Lim, E. C., Ed.; Academic Press: New York, 1982; Vol. 6, pp 1- 95.
(2) Kohler, B. E. *Chem. ReV.* **1993**

(26) Buma, W. J.; Kohler, B. E.; Shaler, T. *J. Chem. Phys.* **1992**, *96*, 399.

(27) Choi, Y. S.; Kim, T. S.; Petek, H.; Yoshihara, K.; Christensen, R. L. *J. Chem. Phys.* **1994**, *100*, 9629- 9271.

(28) Petek, H.; Bell, A. J.; Choi, Y. S.; Yoshihara, K.; Tounge, B. A.; Christensen R. L. *J. Chem. Phys.* **1995**, *102*, 4726- 4739.

(29) Pfanstiel, J. F.; Pratt, D. W.; Tounge, B. A.; Christensen, R. L. *J. Phys. Chem.* **1999**, *103*, 2337.

(30) Bondarev, S. L.; Knyukshto, V. N. *Chem. Phys. Lett.* **1994**, *225*, 346- 350.

(31) Andersson, P. O.; Bachillo, S. M.; Chen, R. L.; Gillbro, T. *J. Phys. Chem.* **1995**, *99*, 16199- 16209.

(32) Jansen, F. J. H. M.; Lugtenburg, J. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser-Verlag: Basel, Switzerland, 1996; Vol. II, pp 233- 258.

(33) Jansen, F. J. H. M.; Kwestro, M.; Schmitt, D.; Lugtenburg, J. *Recl. TraV. Chim. Pays-Bas* **1994**, *113*, 552- 562.

(34) Savitsky, A.; Golay, M. J. E. *Anal. Chem.* **1964**, *36*, 1727.

(35) Steiner, J.; Termonia, Y.; Deltour, J. *Anal. Chem.* **1972**, *44*, 1906.

(36) Christensen, R. L.; Kohler, B. E. *Photochem. Photobiol.* **1973**, *18*, 293- 301.

(37) Hemley, R.; Kohler, B. E. *Biophys. J.* **1977**, *20*, 377- 382.

(38) DeCoster, B.; Christensen, R. L.; Gebhard, R.; Lugtenburg, J.; Farhoosh, R.; Frank, H. A. *Biochim. Biophys. Acta* **1992**, *1102*, 107- 114.

(39) Andersson, P. O.; Gillbro, T.; Asato, A. E.; Liu, R. S. H. *J. Lumin.* **1992**, *51*, 11- 20.

(40) Englman, R.; Jortner, *J. Mol. Phys.* **1970**, *18*, 145.

(41) Turro, N. J. *Molecular Photochemistry*; Benjamin/Cummings: Menlo Park, CA, 1978.