J. Am. Chem. Soc. **1981**, *103*, 7433–7440

Vibrational Spectroscopy of the Electronically Excited State. 4. Nanosecond and Picosecond Time-Resolved Resonance Raman Spectroscopy of Carotenoid Excited States

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Abstract: Resonance Raman and electronic absorption spectra are reported for the S_0 and T_1 states of the carotenoids β-carotene, zeaxanthin, echinenone, canthaxanthin, dihydroxylycopene, astaxanthin, decapreno(C_{50})-β-carotene, β-apo-8′-carotenal, and ethyl β-apo-8′-carotenoate. The results reveal qualitatively similar ground-state spectra and similar frequency shifts in all observed resonance Raman modes between S_0 and T_1, regardless of carotenoid structure. Examinatiion of the relationship of the putative C—C and C═C frequencies in S_0 and T_1 reveals anomalous shifts to lower frequency in the "single-bond" mode upon electronic excitation. These shifts may be due to molecular distortions in the excited state which force changes in molecular motions comprising the observed modes. However, another possibility requiring no distortion is that the interaction (off-diagonal) force constants connecting the C—C and C═C modes change sign upon electronic excitation. This latter phenomenon may provide a unitary explanation for the "anomalous" frequency shifts in the C—C and C═C modes, both in the T_1 states of carotenoids and in the S_1 states of simpler polyenes, without postulating large, unpredicted structural changes upon excitation or general errors in existing vibrational or theoretical analyses. Resonance Raman and absorbance studies with 35-ps time resolution suggest that S_1 lifetime (of the ^1B_u and/or the $^1A_g{}^*$ states) of β-carotene in benzene is less than 1 ps.

The carotenoid pigments play an important role in photobiology, participating in a wide variety of functions including vision and photosynthesis.[2] Characterization of the electronically excited states of the carotenoids is crucial to understanding the photochemical and photophysical mechanisms by which these pigments operate. Quite recently we have developed techniques for recording spontaneous resonance Raman spectra of electronically excited states in fluid solution,[3] and we have reported the time-resolved resonance Raman (TR^3) spectra of several excited-state systems.[4-6] In a preliminary communication,[4] we presented a TR^3 study of the vibrational spectrum of the triplet state of one carotenoid, *all-trans-β*-carotene. In that paper, we reported three vibrational bands due to the pulse radiolytically generated T_1 state of β-carotene. These excited-state vibrations were correlated with the three most intense ground-state resonance Raman peaks.[7-11] We noted that the pattern of vibrational shifts apparently did not agree with the predictions based on molecular orbital calculations[12] and commonly accepted ground-state vibrational assignments, especially in the putative "single-bond-stretch" frequency region. Subsequently, Jensen et al. verified our observations[13] and, by virtue of higher triplet yield due to greater radiolysis pulse intensity, were able to observe additional features in the excited-state resonance Raman spectrum of β-carotene.

In this paper, we extend our TR^3 study of carotenoid triplet states to include several systems related to β-carotene (Figure 1). These compounds are echinenone, zeaxanthin, canthaxanthin, and astaxanthin, which are terminal ring-substituted β-carotenes, decapreno-β-carotene ($C_{50}H_{68}$), a longer chain homologue of β-carotene, dihydroxylycopene, which has no terminal rings, and β-apo-8′-carotenal and ethyl β-apo-8′-carotenoate, which have one terminal ring and one terminal carbonyl functional group. The latter compounds are longer polyene chain homologues of *all-trans*-retinal, the chromophoric unit of the visual pigment rhodopsin. These species embody systematic structural variations from β-carotene and thus provide a more extensive view of the excited-state vibrational spectroscopy of polyenes and the relationship of the spectroscopic parameters to molecular structure.

Experimental Section

Samples of the carotenoids examined in this study were kindly provided by Dr. Percy Manchand. Solutions of 10^{-4} M carotenoid with 10^{-2} M naphthalene or anthracene in spectrograde benzene or THF were freshly prepared prior to use and were exhaustively deoxygenated by bubbling with argon.

Carotenoid triplet states were produced by two method: (a) by using pulse radiolysis, with naphthalene acting as a triplet sensitizer; and (b) by optical excitation of a sensitizer (anthracene) and subsequent energy transfer. In the radiolysis experiments, electron pulses were provided by a 4-MeV van de Graaf generator operating at a repetition frequency of 6 Hz and a 800-ns pulse width. A special quartz cell with four optical faces was constructed to allow the radiolysis beam to strike the sample from above (*z* direction) and permit either transient absorption (*x* direction) or resonance Raman (*x* direction, laser excitation; *y* direction, scattered light collection) experiments to be done without changing optical geometry (Figure 2). Solutions were flowed through the cell from a syringe drive such that each radiolysis pulse irradiated a fresh portion of solution. Under these conditions initially produced excited states (S_1 and T_1) of benzene are rapidly converted by energy transfer and intersystem crossing to naphthalene (T_1). With 3×10^{-5} M of carotenoid present energy transfer to carotenoid (T_1) is complete within ca. 2 μs.[14]

Transient absorption spectra and kinetics were recorded by using standard fast reaction techniques.[15] A xenon lamp source interrogated the solution before, during, and after the radiolysis pulse. The time

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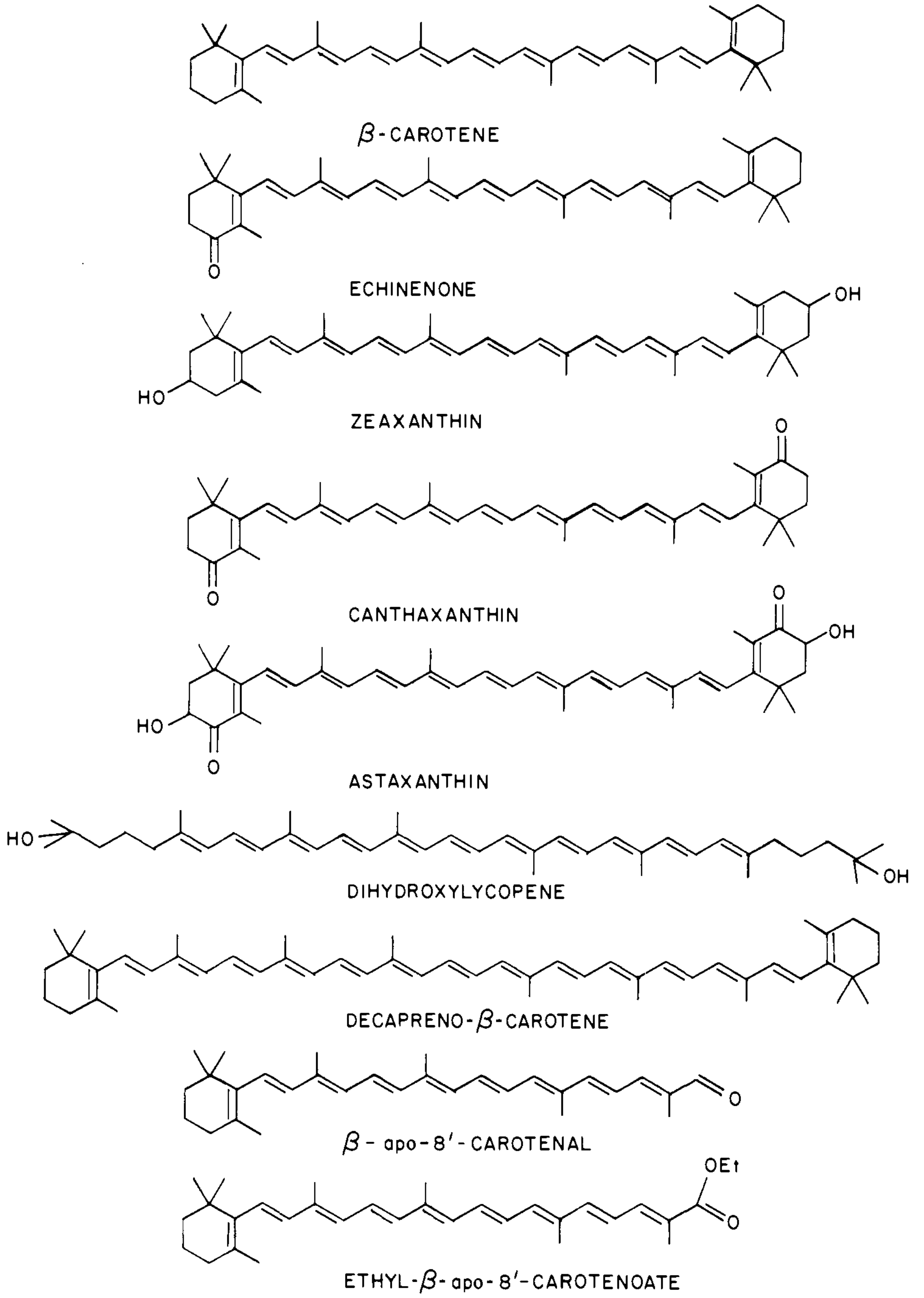

Figure 1. Structures of the carotenoids examined in the present study.

interval during which the sample was exposed to the monitoring source was restricted to ca. 10 ms by a programmable fast shutter. The beam was passed through a high-intensity monochromator and detected by a photomultiplier tube. The transient absorption data were processed with a PDP-11/34 computer which calculated the triplet-triplet absorption spectra and triplet decay kinetics from the transmittance vs. time input data at a series of different wavelengths.

The time-resolved resonance Raman excitation source for the radiolysis experiments was the second harmonic (531.8 nm) of a pulsed Nd:YAG laser (Quanta-Ray DCR-1). The 7-ns laser pulse was initiated by a timing circuit trigger such as to arrive at the sample approximately 2 μs after the electron beam pulse, when the transient concentration of carotenoid triplets was near a maximum. The Raman scattering was collected at 90°, dispersed in a 0.5-m spectrograph, and detected with a PAR 1205I ISIT vidicon. The vidicon signal was processed by an optical multichannel analyzer (PAR OMA-I) and recorded on an X-Y plotter. The image intensifier stages of the vidicon were gated such that they were on during the laser pulse to collect the Raman signal and off during the radiolysis pulse to avoid collecting the intense Cerenkov radiation, which is emitted when the electron beam strikes the sample. Resonance peaks of the solvent and the ground-state carotenoids were used to calibrate the triplet Raman frequencies. Estimated error in the triplet frequencies is ± 2 cm^{-1}.

The sample syringe had sufficient capacity to flow the solution through the observation cell continuously for $\simeq 1$ min; therefore this represented the maximum time over which signal could be accumulated. Satisfactory TR^3 spectra could be collected by using moderate ($\simeq 3$ mJ/pulse) laser intensities and 15-s spectral accumulation times (90 laser pulses).

The optically excited sensitizer experiments required temporal delay between the excitation and probe laser pulses similar to that required between the electron pulse and the laser pulse in the radiolysis experiments, and the reasons for the required delay were similar. The excitation pulse (Nd:YAG third harmonic, 354.7 nm) creates anthracene triplets, which then must transfer their energy to the carotenoid in a bimolecular process before the carotenoid triplets can be observed. At typical concentrations of carotenoid and anthracene, this reaction was complete in ca. 1 μs. The required delay between excitation and probing was generated in two ways: in early experiments, by optical delay; and later, by dual laser synchronized pulse-probe techniques. The apparatus is shown in Figure 3. Optical delay was barely usable in these systems;

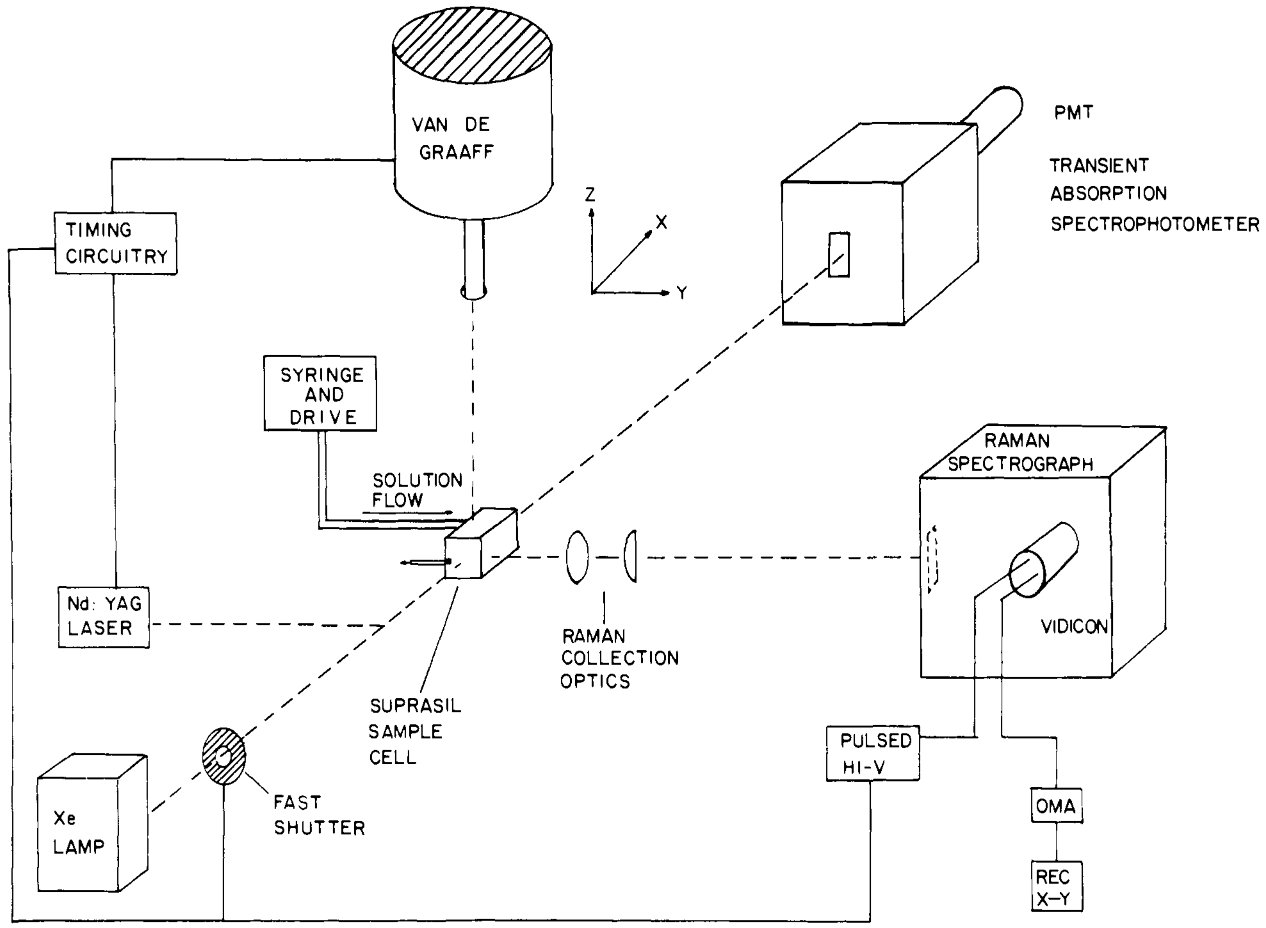

Figure 2. Simplified diagram of the experimental apparatus for the pulse radiolysis/time-resolved resonance Raman experiments.

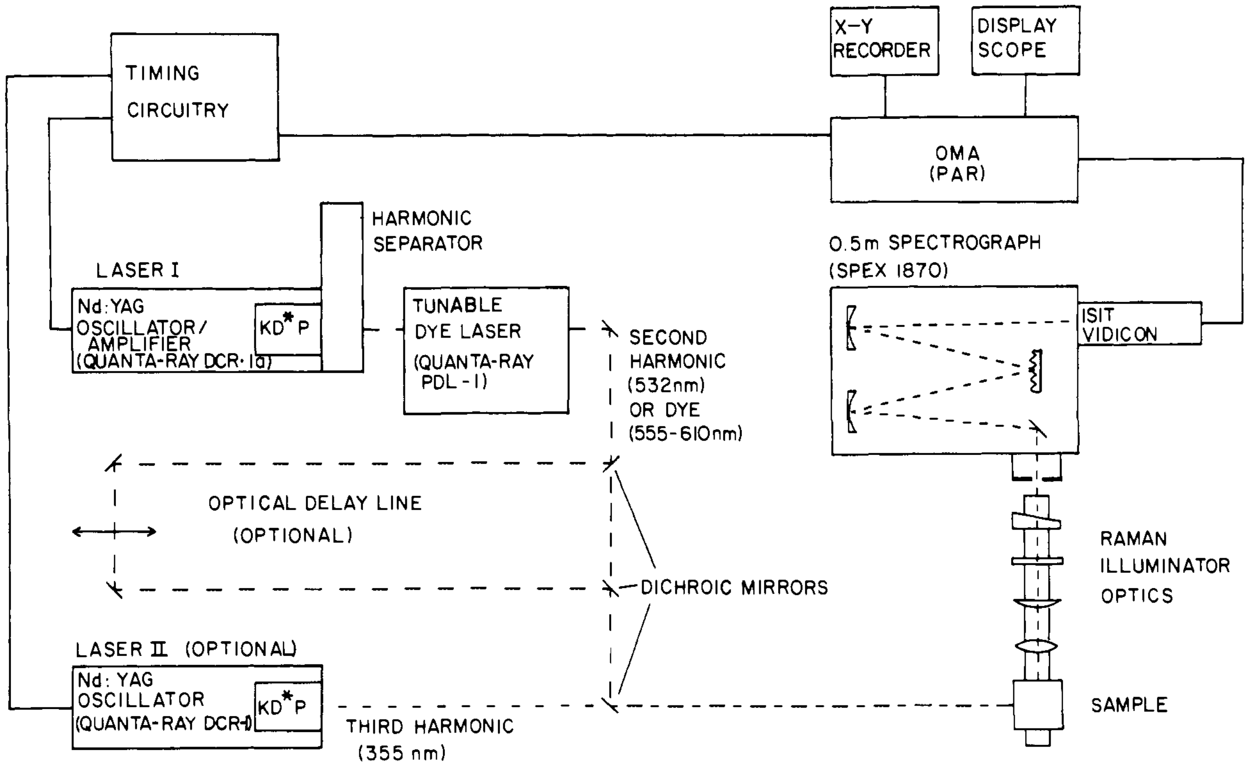

Figure 3. Simplified diagram of the experimental apparatus for the optically sensitized triplet generation/TR^3 studies. Requisite time delays were obtained either by optical delay or by the two-laser experimental configuration (see text). The optical delay line is not to scale; its actual length was ca. 120 ft.

we were able to acquire spectra of the canthaxanthin triplet (with an optical delay line 120-ft long!,) but even this extreme optical delay did not allow the bimolecular energy-transfer reaction to proceed to a sizable fraction of completion. The microsecond delays which were needed to optimize this experiment required separate lasers for the excitation and probe pulse.

Table I. Electronic Absorption Spectra ($S_1 \leftarrow S_0$ and $T_2 \leftarrow T_1$) and Triplet (T_1) Lifetimes of Carotenoids[a]

carotenoid	$S_1 \leftarrow S_0$ absorption λ_{max},[b] nm	$T_2 \leftarrow T_1$ absorption λ_{max}, nm	T_1 lifetime, μs
β-carotene	438, 464, 493	526[c] (bz)	6.3[d] (bz)
		510[d] (hex)	5.0[d] (hex)
echinenone	464	550[b] (bz)	3.4[b] (bz)
zeaxanthin	435 (sh), 457, 491	520[b] (bz)	6.5[b] (bz)
		505[c] (hex)	
canthaxanthin	473	555 (bz)	3.8 (bz)
astaxanthin	478	580 (bz)	6.2 (bz)
dihydroxylycopene	450, 477, 511	540[b] (bz)	5.3[b] (bz)
		525[f] (hex)	5.0[f] (hex)
decapreno-β-carotene	523	590 (bz)	6.5 (bz)
β-apo-8′-carotenal	440 (sh), 470, 495(Sh)	520[d,e] (hex)	6.9[d] (hex)
ethyl β-apo-8′-carotenoate	430 (sh), 456, 480	508[c,e] (hex)	5.8[d,g] (hex)

[a] bz = benzene solvent; hex = hexane solvent; sh = shoulder. [b] Benzene solvent; present work. [c] Reference 20. [d] Reference 21. [e] Reference 22. [f] Values for *all-trans*-lycopene. [g] Value for methyl ether.

The carotenoids examined by this method included canthaxanthin, astaxanthin, and decapreno-β-carotene. Each of these carotenoids have their $T_1 \rightarrow T_2$ absorption at such long wavelength that the Nd:YAG second harmonic (the only probe wavelength available in the radiolysis experiments) provided inadequate resonance enhancement; a tunable dye laser probe pulse was necessary. Probe wavelengths were 555 nm (canthaxanthin), 602 nm (astaxanthin), and 602 nm (decapreno-β-carotene). A Quanta-Ray PDL-1 YAG-pumped dye laser was used with Rhodamine 590 (555 nm) or Rhodamine 640 (602 nm). The previous β-carotene triplet results obtained by pulse radiolysis were repeated by using the optical excitation method described above, and the results were confirmed to be identical. Improved signal-to-noise ratios could be achieved because more extensive signal accumulation was possible. Triplet frequencies were calibrated as described above for the radiolysis experiments. The frequency precision was ± 2 cm^{-1}.

The general data collection scheme for the triplet state TR^3 spectra was as follows: the ground-state resonance Raman spectrum was collected into the B channel of the OMA with the excitation (electron or laser) pulse off. Under the same conditions, the spectrum with the electron beam or excitation pulse on was recorded in the OMA A channel. This TR^3 spectrum contains both excited-state peaks and peaks due to remaining ($\simeq 90\%$) ground state. Subtraction of the properly normalized channel B (ground-state) spectrum from channel A gave the excited triplet state TR^3 spectrum. The normalization criterion for the subtraction was cancellation of the solvent and ground-state peaks.

The vidicon Raman spectrograph used to record picosecond TR^3 spectra is the same unit described above for the nanosecond experiments. The only modification was the addition of a circuit to synchronize the scanning of the OMA detector with the low repetition rate of the mode-locked Nd:YAG laser. This is necessary to minimize noise caused by long vidicon accumulation times. The laser employed was a Quantel NG50 system, composed of a Nd:YAG oscillator and first-stage amplifier and a Nd:glass second-stage amplifier. The oscillator was passively mode locked and equipped with a pulse extractor which allowed the laser to deliver either a single-mode-locked pulse of 34 ± 5-ps duration or a train of eight to ten such pulses within the Q-switched pulse width (approximately 40 ns) of the oscillator. With use of the first amplifier stage only, pulses of 2-mJ typical energy at the Nd:YAG second harmonic could be produced at a repetition rate of 0.5 Hz. With the second-stage amplifier operating, maximum per pulse energy at 532 nm was 30 mJ at a maximum repetition rate of 1 pulse/30 s. The temporal characterizations of the mode-locked pulses were measured with a Hammamatsu C979/RKB streak camera system.

Ground-state absorption spectra (Cary 14) and resonance Raman spectra (Spex monochromator, photon counting, 514.5-nm CW Ar^+ excitation) were also acquired for each compound.

Results

Absorption spectra data for the carotenoid ground and triplet states are presented in Table I. The visible absorption spectra of the ground-state molecules generally consist of three vibronic bands with varying degrees of resolution at room temperature. Echinenone, for example, shows only two very weak shoulders on either side of the central peak, while dihydroxylycopene shows three distinct, well-resolved peaks. The wavelengths of the central peaks of all of the ground-state compounds range from 456 to 477 nm.

Attempts were made to observe TR^3 spectra of the S_1 state of β-carotene by using 35-ps pulses from a mode-locked Nd:YAG laser. Illumination conditions were such that a singlet state having a lifetime of 1 ps would have been 50% saturated (25% populated). The RR spectra obtained showed peaks due to solvent and ground-state β-carotene but none due to excited states of β-carotene (unless the ground and S_1 states have identical RR frequencies and intensities, a most unlikely circumstance). Signal-to-noise ratio in these spectra was such that a peak at 10% of the intensity of the ground-state β-carotene peaks would have been observable. Thus we conclude that the S_1 (^1B_u) state of β-carotene in benzene has a lifetime much shorter than 1 ps. This conclusion is supported by transmittance experiments, which show the same optical density of a β-carotene solution in a spectrophotometer cuvette whether the illumination source is a spectrophotometer tuned to 532 nm, a CW Kr^+ laser operated at 530.9 nm (corrected for wavelength), a Q-switched Nd:YAG laser producing 7-ns pulses at 532 nm, or a mode-locked Nd:YAG laser producing 35-ps pulses at 532 nm. These results show that saturation behavior (ground-state depletion) is absent in the absorption spectrum of β-carotene, even when illuminated by 2-mJ pulses of 35-ps duration. This suggests that both the ^1B_u and $^1A_g{}^*$ states have lifetimes shorter than 1 ps. This work is described in more detail elsewhere.[16,17]

The triplet–triplet absorption maxima are shown in the third column of Table I. The values we determined were for benzene solutions, while the literature references cited for several species were for hexane solutions. It appears from the β-carotene, zeaxanthin, and lycopene data that the T–T maxima in benzene are red-shifted by approximately 15 nm from the hexane maxima. Thus, the T–T maxima for six of the carotenoids in benzene solutions span the 520–550-nm wavelength range, providing good resonance conditions for probing the triplet states of these molecules with the 531.8-nm Nd:YAG laser line. Carotenoids canthaxanthin, astaxanthin (3,3′-dihydroxy′β,β-carotene-4,4′-dione), and decapreno-β-carotene ($C_{50}H_{68}$) have their T–T absorption maxima at wavelengths too long (>560 nm) to provide sufficient resonance Raman intensity enhancements when interrograted at 531.8 nm; therefore pulsed dye laser interrogation was necessary (see Experimental Section).

The triplet states of the carotenoids studied have lifetimes ranging from 3 to 6 μs. The bimolecular formation of the carotenoid triplets from the naphthalene precursor has a half-life of 1–2 μs under our concentration conditions. Therefore, the carotenoid triplet concentration reaches its maximum approximately 2–4 μs after the end of the radiolysis or laser excitation pulse. The timing circuitry which controlled our experiments was set to deliver the laser pulse (and turn on the image intensifier high voltage to the vidicon) 2 μs after the end of the radiolysis pulse. This ensured that the TR^3 spectrum was taken near the maximum triplet concentration. Ground-state and triplet resonance Raman spectra of canthaxanthin obtained by using the vidicon spectrograph are

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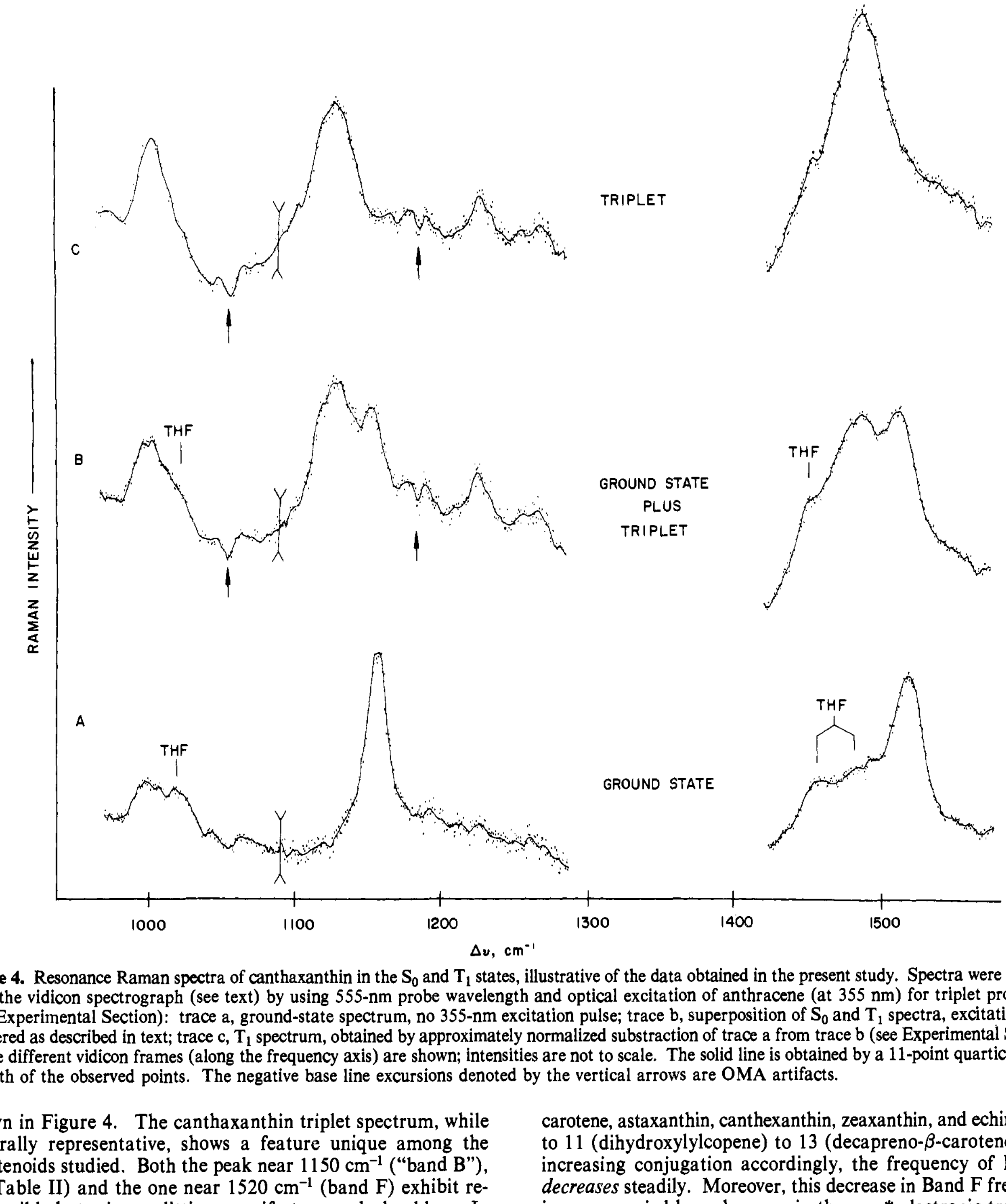

Figure 4. Resonance Raman spectra of canthaxanthin in the S_0 and T_1 states, illustrative of the data obtained in the present study. Spectra were obtained with the vidicon spectrograph (see text) by using 555-nm probe wavelength and optical excitation of anthracene (at 355 nm) for triplet production (see Experimental Section): trace a, ground-state spectrum, no 355-nm excitation pulse; trace b, superposition of S_0 and T_1 spectra, excitation pulse delivered as described in text; trace c, T_1 spectrum, obtained by approximately normalized substraction of trace a from trace b (see Experimental Section). Three different vidicon frames (along the frequency axis) are shown; intensities are not to scale. The solid line is obtained by a 11-point quartic running smooth of the observed points. The negative base line excursions denoted by the vertical arrows are OMA artifacts.

shown in Figure 4. The canthaxanthin triplet spectrum, while generally representative, shows a feature unique among the carotenoids studied. Both the peak near 1150 cm^{-1} ("band B"), see Table II) and the one near 1520 cm^{-1} (band F) exhibit reproducible but minor splitting, manifest as weak shoulders. In the case of band B of canthaxanthin, this has been noted by other workers (13b).

The numerical resonance Raman data are given in Table II. The ground-state spectra of all of the carotenoid species consist of intense features at $\sim$1520 and $\sim$1150 cm^{-1}, with medium-to-weak bands at $\simeq$1000, $\simeq$1190, $\simeq$1210, and $\simeq$1270 cm^{-1}. The intense bands have generally been assigned to the in-phase double-bond stretching vibration of the conjugated chain ($\simeq$1520 cm^{-1}) and the in-phase single-bond stretching motion ($\simeq$1150 cm^{-1}),[7-12] while the previous assignments of the other observed features have been more varied.

The decrease of the frequency of the ground-state peak at 1500–1530 cm^{-1}, which is commonly taken to be the C=C stretch, with increasing *trans*-polyene chain length is consistent with the C=C assignment for this mode. Our data show (band F, Table II) that, as the number of trans C=C double bonds increases from 8 (β-apo-8′-carotenal and ethyl β-apo-8′-carotenoate) to 9 (β-carotene, astaxanthin, canthexanthin, zeaxanthin, and echinenone) to 11 (dihydroxylylcopene) to 13 (decapreno-β-carotene), thus increasing conjugation accordingly, the frequency of Band F *decreases* steadily. Moreover, this decrease in Band F frequency is accompanied by a decrease in the π–π^* electronic transition energy in a manner that is now widely recognized[11,18,19] to connote increased π delocalization. Indeed, our ground-state band F frequencies and electronic transition energies are directly superimposable upon the $\nu_{C=C}$ vs. $1/\lambda_{max}$ plots previously constructed by Clark et al.[19] Salares et al.,[18] and (after appropriate corrections) Rimai et al.[12]

We note that, in the ground state, there is a positive linear correlation between the frequencies of the putative C—C stretch

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Table II. Resonance Raman Frequencies (cm^{-1}) of the Ground (S_0) and Triplet (T_1) States of Carotenoids

	band A		band B[b]		band C		band D		band E		band F[c]	
carotenoid	S_0 T_1	Δ[a]	S_0 T_1	Δ	S_0 T_1	Δ	S_0 T_1	Δ	S_0 T_1	Δ	S_0 T_1	Δ
β-carotene	1003 *1014*	+11	1157 *1126*	−31	1190 *1188*[d]	−2	1211 *1236*[d]	+25	1266		1521 *1498*	−23
echinenone	1004 *1011*	+7	1156 *1127*	−29	1192		1213		1272		1521 *1493*	−28
zeaxanthin	*1014*		1156 *1123*	−33	1190		1212		1266		1522 *1501*	−21
canthaxanthin	1003 *1011*	+8	1155 *1129* (1119)	−26	1192		1214 *1225*	+11	1270		1519 *1491* (1484)	−28
astaxanthin	1005 *1016*	+11	1155 *1128*	−29	1192		1215		1277		1519 *1493*	−26
dihydroxylycopene	1001 *1007*	+6	1153 *1127*	−26	1196		1214		1280		1511 *1490*	−21
decapreno-β-cartene			1151 *1124*	−26	1194		1212		1283		1504 *1484*	−27
β-Apo-8′-carotenal	*1014*		1158 *1134*	−24	1183		1213		1269		1527 *1512*	−15
ethyl β-apo-8′-carotenoate	*1012*		1158 *1138*	−20	1193 *1185*	−8	1209		1274 *1257*	−17	1528 *1510*	−18

[a] Δ = (T_1 frequency) − (S_0 frequency). [b] Putative in-phase C-C stretch (see text). [c] Putative in-phase C=C stretch (see text). [d] T_1 β-carotene frequencies from ref 13.

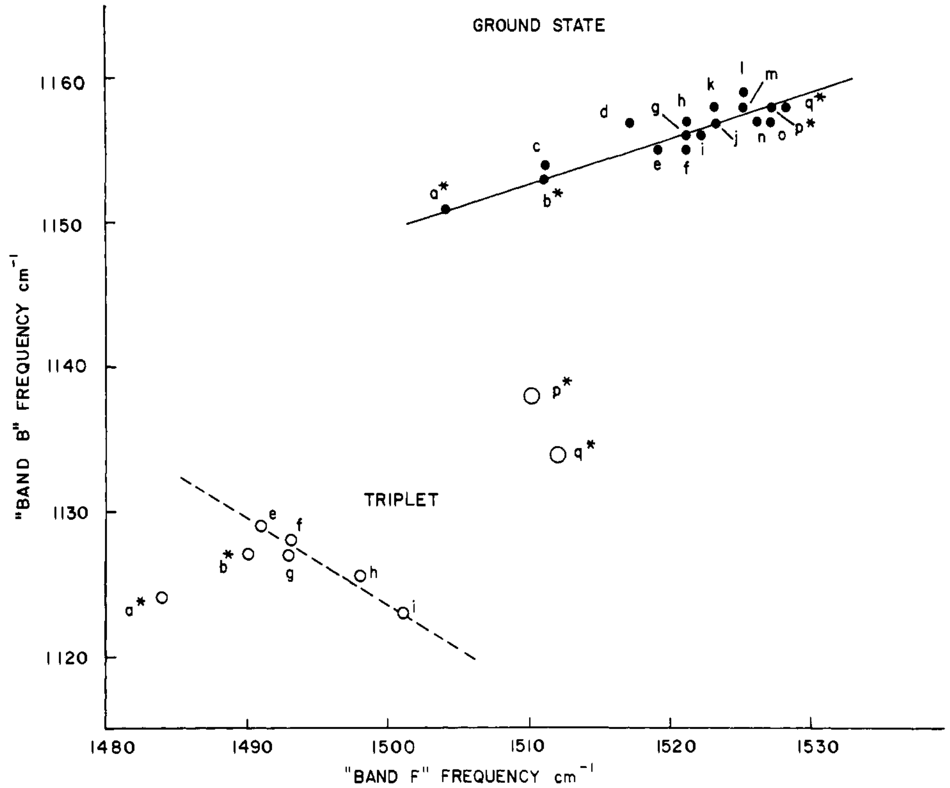

Figure 5. Plots of resonance Raman "C—C stretch" frequency (band B, see text) vs. "C═C stretch" (band F) for all carotenoids of the present study and others for which the required frequencies have been reported: filled circles, ground-state frequencies; open circles, triplet state frequencies. All ground-state points are from this study except as noted below. All triplet points are from this study. Figure legend: (a) decapreno-β-carotene; (b) dihydroxylycopene; (c) ovorubin;[19] (d) *P. insularium* astaxanthin protein;[19] (e) canthaxanthin; (f) astaxanthin; (g) echinenone; (h) β-carotene; (i) zeaxanthin; (j) astaxanthin;[19] (k) canthaxanthin, astaxanthin;[18] (l) ovoverdin;[19] (m) echinenone, cryptoxanthin;[18] (n) zeaxanthin;[18] (o) lutein;[18] (p) β-apo-8′-carotenal; (q) ethyl β-apo-8′-carotenoate. The asterisks following the letters a, b, p, and q denote structures other than β-carotene derivatives, i.e., having other than nine all-trans conjugated C═C bonds and two terminal cyclohexene rings.

(band B, Table II) and the C═C stretch (band F). The ground-state frequencies for numerous other carotenoids reported by other workers[12,18,19] also fall on the linear plot of band B vs. band F, regardless of structure, protein environment, etc. This correlation is shown in Figure 5 (ground state, filled circles). Inasmuch as the C═C frequency is reliably correlated (inversely) with the extent of polyene delocalization,[12,18,19] this relationship shows that *both* of these vibrational frequencies share an inverse relationship to the extent of delocalization of the carotenoids. This is expected for the C═C stretch, as noted above, but not to a first approximation for the C—C stretch, the primary force constant of which should *increase* directly with increasing conjugation.

The excited-state resonance Raman frequencies are also in Table II along with the frequency differences observed between the ground and excited states. We estimate that 5–10% of the carotenoid molecules were promoted to the triplet state; thus, even with the improved resonance condition at 531.8 nm in the excited state vs. the ground state, we were generally able to observe only bands A, B, and F in the radiolytically excited triplet spectra. (Jensen et al.[13] report three additional bands in the β-carotene triplet. We observe these bands in the optically excited spectra, wherein more signal averaging is possible.) The lone exception to this rule was the detection of weak features in the $\simeq 1200\text{-cm}^{-1}$ region for ethyl β-apo-8′-carotenoate and canthaxanthin.

The first observation to be made from the excited-state vibrational data is that qualitatively similar frequency shifts occur between the ground and T_1 states of *all* of the carotenoids studied, despite the structural differences among the various systems. Band A shifts $\simeq 5\text{–}10\ \text{cm}^{-1}$ to higher frequency in the excited state, while bands B and F both decrease by $\simeq 20\text{–}30\ \text{cm}^{-1}$, with the shift in band B at least as large as, generally larger than, the shift in band F. This, as we pointed out in our earlier work,[4] appears inconsistent with a simple assignment of band B as C—C stretch in both the ground and triplet states. If such an assignment were correct, it may be argued on the basis of expected changes in primary force constants of the C—C and C═C bonds that increased conjugation in the π–π^* excited state should cause band B to *increase* in frequency between the ground and T_1 states while the double-bond frequency (as observed) decreases.

The second major result of the triplet TR[3] study is presented in Figure 5, which shows the dependence of band B frequency upon band F for the nine carotenoid triplets examined (open circles). These fall into two groups: on the one hand, the five β-carotene derivatives which have nine all-trans C═C bonds and terminal cyclohexane rings; and on the other hand the "other" structures including decapreno-β-carotene, dihydroxylycopene, and the two retinal homologues. No differences among these structures are apparent in the ground-state correlation in Figure 5. Apparently the potential energy distribution of band F in the T_1 state is more sensitive to molecular structure than it is in the ground state. In particular, the band F frequency of the T_1 state of the retinal homologues appears to contain a substantial contribution from C═O stretch, and therefore band F is anomalously high in frequency for these two triplets compared to the others. The two carotenoids with longer all-trans C═C conjugated chains, dihydroxylycopene and decapreno-β-carotene, have anomalously low T_1 band F frequencies consonant with increased delocalization above that observed in the β-carotene-like structures. Structural sensitivity of excited-state *electronic* energies, which may arise similarly, has been indirectly noted in previous work[15] wherein retinal fails to follow the same C═C frequency vs. $1/\lambda_{\max}$ correlation as other carotenoids, and explicitly noted[18] in studies of aggregation phenomena.

As is the case in the ground state, the five "main-sequence" carotenoid triplets exhibit a linear frequency correlation of band B with band F. The slope of this triplet correlation, however, is the *opposite* of that observed for the ground state (dashed line, Figure 5). That is, the T_1 band B frequency *increases* with *decreasing* band F frequency, essentially as expected from simple π delocalization and primary force constant arguments.

Discussion

The present theory and available data on polyene (including carotenoid) ground and excited states support several views, all of which are individually reasonable but some of which appear mutually contradictory. First, the enormous intensity of the resonance Raman peaks that we designate band B and band F suggests that these vibrational modes represent the dominant fraction of the molecular motions having greatest Franck–Condon activity with respect to the $\pi \rightarrow \pi^*$ electronic transitions. The Franck–Condon active motions are widely believed to be the in-phase C—C and C═C stretches. Second, numerous theoretical calculations of excited-state structures of polyene singlets and triplets, using various theoretical approaches and widely varying degrees of sophistication, are in complete accord concerning one point: between the ground and excited states, the single bonds shorten and the double bonds lengthen. That is, the single-bond primary force constants increase and the double-bond primary force constants decrease. Third, ground-state vibrational analyses are generally in accord with the simple assignments of bands B and F suggested by the resonance Raman intensities[7-10] (vide supra), although most of these analyses show band B to be somewhat mixed and one analysis[11] suggests that, despite the resonance Raman evidence, the C—C stretch potential energy is distributed almost equally among band B and at least three other modes. Fourth, vibronically resolved fluorescence excitation studies of several polyenes[23-26] reveal that, in the excited singlet state, the vibrational frequency ascribed to C═C stretch *increases* relative to ground state despite the general theoretical agreement that the primary C═C force constant must *decrease* in S_1 relative to S_0. Although mixing with the C—C motion (which increases in frequency between S_0 and S_1) has been suggested to explain this effect,[27] it appears most unlikely that the single bond contribution to a mode at $\sim 1600\ \text{cm}^{-1}$ could be large enough to overcome the effect of increased conjugation in S_1 relative to S_0 and the decrease in net π bond order in the $\pi \rightarrow \pi^*$ excited state, both of which must act to diminish the primary C═C force constants. Finally, our present data on the carotenoid triplets show equally unexpected but opposite behavior to the polyene singlets, that is, *both* the "single-bond stretch" and the "double-bond stretch" appear at *lower* frequency in T_1 relative to S_0. Again, this cannot be explained simply by mixing of the C═C motion into the normal mode observed as band B (the putative C—C stretch), because the downward shifts in band B between S_0 and T_1 are as large as or larger than those of band F.

There are several ways to rationalize the present data concerning polyene vibrational and electronic states, by postulating either current ground-state vibrational assignments to be in error, theoretical predictions of excited-state force constants or structures to be seriously inadequate, extreme excited-state structural distortions which effectively change vibrational assignments between S_0 and S_1 or T_1, or unexpected excited-state resonance Raman enhancement such that the intense modes in the ground state are not similar motions to those in the excited state. We suggest an alternative, qualitative explanation which requires none of these drastic postulates, has experimental precedent in small-molecule photophysics, is susceptible to quantitative evaluation, and is indeed supported by the experimental evidence in the present work. Our suggestion is that the interaction (off-diagonal) force constant connecting the primary force consants for the C—C and C═C stretches, and perhaps other interaction constants (e.g., involving C—H wag), may *change sign* between S_0 and S_1 or T_1 in polyenes. This effect, together with expected changes in magnitude of primary and interaction constants, may account for the superficially "anomalous" shifts in the S_1 C═C frequency and the T_1 band B, relative to the S_0 values.[28]

Experimental precedents for this suggestion exist in small molecule spectroscopy, wherein it is known that the stretch–stretch interaction constants of CO_2^+[29] and BO_2,[30] and the ortho and meta stretch–stretch interaction constants and several stretch–bend interaction constants of benzene,[31] change sign upon electronic excitation. The sign changes of the interaction force constants are interpreted in terms of the nodal properties of the excited electronic states involved compared to the ground states.[32] In the case of the polyenes, the energetic proximity in both the singlet and triplet manifolds of excited states having B_u and A_g symmetry may lead to unusual polarization effects in S_1 and T_1 and result in interaction force constants different in sign from those in S_0.

While it has often been recognized that interaction force constants are important in polyene systems[11,25,26,33,34] and that their

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magnitude may be large and sensitive to electronic structure,[25-27,35] it does not appear feasible for simple changes in the magnitude of these interactions to result in the vibrational shifts observed between polyene S_0 and S_1 or T_1, for reasons discussed above. A change in the *sign* of the interaction constants, however, might result in the observed shifts. Attempts to determine whether the observed ground-state and excited-state frequencies can be modeled successfully with reasonable values of primary and interaction force constants are underway in these laboratories. (See Note Added in Proof.)

The frequency correlation shown in Figure 5 may be taken as evidence that the proposed change in sign of the stretch–stretch interaction indeed occurs. The frequency of band B for the β-carotene derivatives is directly and positively correlated (slope equals +0.3) with that of band F in the ground state, while in the T_1 state the correlation is negative (slope equals −0.6). If the molecular motions represented by band B and F are similar between S_0 and T_1 and if the primary C—C and C=C force constants respond in the predicted manner to changes in conjugation within a given electronic state, this change in slope is direct evidence for a change in sign of the relevant interaction constants in T_1 compared to the case for S_0.

The structural implications of our interpretation of the carotenoid S_0 and T_1 results are that the commonly accepted view of the effect of electronic excitation upon C—C and C=C distances is correct, and that no other "major" changes in the polyene backbone structure (i.e., sufficient to change substantially the molecular motions represented by the observed vibrational modes) need occur. Specifically, the change in torsional angle between the cyclohexane ring and the backbone plane of retinal, predicted by Warschel and Karplus,[12] is entirely premissible within the framework of our results because lycopene (which has its cyclohexene ring opened and all its C=C bonds trans) shows essentially the same pattern of backbone modes as the β-carotene derivatives. On the other hand, large backbone rearrangements such as the rotation around the 15,15′ C=C bond of β-carotene proposed by Jensen et al.[13] and the localization of the triplet electron density in two allyl-like units, effectively dividing the polyene chain into three units, proposed by Lafferty *et al.*[36] are not required in our interpretation and indeed are somewhat inconsistent with the behavior of the observed C=C frequencies of the triplets. That is, any effect which interrupts the all-trans, delocalized polyene backbone structure should result in upshifted C=C frequencies. The $S_0 \rightarrow T_1$ shift of the 1236-cm^{-1} (T_1) peak of β-carotene is taken by Jensen et al. as evidence for large torsional motion around the 15,15′ C=C bond in the excited state.[13] We cannot conclusively reject this suggestion. We note, however, that neither β-carotene nor any of the other carotenoids in this study show appreciable cis–trans isomerization from T_1, which argues against such a torsion. We suggest that changes in interaction force constants (as well as primary force constants) can explain this shift without requiring large structural changes. Precedent for this suggestion is seen in the benzene case, wherein several interaction force constants change sign upon electronic excitation without large concomitant alterations in molecular structure.[31]

We may summarize our findings and interpretations as follows. The absence of observable Raman modes due to the S_1 state of β-carotene in our picosecond TR^3 experiment suggests a S_1 (^1B_u) lifetime less than 1 ps for β-carotene in benzene. The absence of saturation effects in the picosecond absorption spectra and the absence of attenuation of ground-state RR intensities in the picosecond TR^3 experiment suggest 1-ps maximum lifetimes for both the ^1B_u and $^1A_g{}^*$ states. All of the carotenoids studied show similar shifts of observed resonance Raman frequencies between S_0 and T_1. The two retinal homologues studied show an anomalously high band F (C=C stretch) frequency in T_1, apparently due to significant contribution of C=O stretch to this normal mode in the excited state. The longer chain carotenoids have anomalously low band F frequencies. The remaining carotenoids having the β-carotene backbone show a change in the sign of the slope of the linear correlation between the "C=C" frequency and the "C—C" frequency, from positive in the ground state to negative in T_1. If the assignment of these modes does not change upon electronic excitation, this is evidence that the interaction force constants connecting the "C—C" and "C=C" motions change sign upon electronic excitation. Such changes in interaction force constants may explain the seemingly anomalous frequency shifts observed between S_0 and both S_1 and T_1 in polyenes, without invoking either large excited-state structural distortions or general error on the part of previous vibrational analyses or theoretical predictions of excited-state properties. Studies aimed at testing our interpretations, both by experiment and by vibrational modeling, are in progress.

Note Added in Proof. Model vibrational calculations (M.-H. Huang and W. H. Woodruff, unpublished results) have been performed on a four-atom C—CH=C system. These calculations show that the observed S_0 and T_1 frequencies of bands A, B, and F, and the qualitative interdependence of the band B and F frequencies in S_0 and T_1, can be reproduced using reasonable primary force constants without changing the signs of interaction force constants. We have not, however, established whether this oversimplified system is an adequate model for the carotenoids (for example, the model obviously lacks conjugation, methyl substitution, and inversion symmetry).

Acknowledgment. We are grateful for support of this work by NSF Grants CHE78-09338 and CHE81-09541 and Robert A. Welch Foundation Grant F-733 (W.H.W.). Work at the Center for Fast Kinetics Research is supported by the Biotechnology Branch of the Division of Research Resources of the National Institutes of Health (Grant RR00886) and by the University of Texas. We are also grateful to Professors J. K. Burdett and B. S. Hudson and Dr. R. Wilbrandt for helpful discussions.

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