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Isolation by High-Pressure Liquid Chromatography of the Cis–Trans Isomers of β-Apo-8′-carotenal. Determination of Their S$_0$-State Configurations by NMR Spectroscopy and Prediction of Their S$_1$- and T$_1$-State Configurations by Transient Raman Spectroscopy

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Abstract: High-pressure liquid chromatography to isolate cis–trans isomers of β-apo-8′-carotenal was developed, and the configurations of nine isomers, i.e. all-trans, 7-cis, 9-cis, 13-cis, 15-cis, 13′-cis, 9,13-cis, 9,13′-cis, and 13,13′-cis isomers, were determined by NMR spectroscopy. The C,H- and H,H-COSY, C,H-COLOC, and H,H-ROESY spectra were recorded to assign the olefin proton signals; each cis–trans configuration was identified by the use of the chemical shifts of the olefinic protons and also by NOE correlation peaks among olefinic and methyl protons. Resonance Raman spectroscopy was applied to the above set of isomers except for 9,13′-cis: (1) The Raman spectra of the isomers, in the S$_0$ state, were recorded to identify the key Raman lines of each cis–trans configuration. (2) The Raman spectra of S$_1$ species generated from the isomers were recorded by a two-color or one-color method using picosecond pulses. The C=C stretching Raman line with an abnormally high frequency showed that the S$_1$ state probed by Raman spectroscopy is actually the $2^1A_g^-$ state, which is vibronically coupled with the S$_0$, $^1A_g^-$ state through this a$_g$-type, C=C stretching mode. The S$_1$ Raman spectra were different from one another, a fact which suggests that each S$_1$ species takes a different configuration and that no isomerization takes place in the S$_1$, $2^1A_g^-$ state. (3) The Raman spectra of T$_1$ species which were generated through intersystem crossing or triplet sensitization were recorded by a picosecond or nanosecond pump-and-probe method. The set of T$_1$ Raman spectra agreed with one another, and analyses by HPLC of triplet-sensitized isomerization showed efficient isomerization from each cis isomer to the all-trans isomer. Both results indicate that extremely efficient isomerization takes place from each cis to all-trans in the T$_1$ state. Parizer–Parr–Pople calculations of the bond order predicted this efficient T$_1$-state isomerization.

Introduction

Carotenoids in photosynthetic systems have dual functions of light harvesting and photoprotection,[1] and a natural selection of the carotenoid configurations has been found in purple photosynthetic bacteria in relation to the above functions: the all-trans configuration is selected by the light-harvesting complex (LHC) for the light-harvesting function, while the 15-cis configuration is selected by the reaction center (RC) for the photoprotective function.[2,3]

As an attempt to reveal the reason for this natural selection, the ground-state and the excited-state properties have been compared among various cis–trans isomers of β-carotene, a prototype symmetric carotenoid: First, a set of cis–trans isomers was isolated by HPLC and their configurations were determined by NMR spectroscopy.[4–6] Second, a set of cis–trans isomers (all-trans, 7-cis, 9-cis, 13-cis, and 15-cis) in the S$_0$ state was examined by NMR[7] and resonance Raman[8] spectroscopy. Third, T$_1$ species which were generated from the set of isomers were examined by time-resolved Raman[9] and time-resolved absorption[10] spectroscopy. Fourth, S$_1$ species generated from the isomers were examined by transient Raman and time-resolved absorption spectroscopy.[11–13] Fifth, direct and triplet-sensitized photoisomerizations as well as thermal isomerizations were analyzed by HPLC.[14] Sixth, in order to explain the S$_0$-state and T$_1$-state isomerization, the carbon–carbon bond orders of a model polyene in the S$_0$ and T$_1$ states were calculated by the Parizer–Parr–Pople (PPP) method including single- and double-excitation configurational interactions (SD-CI).[14]

The results can be summarized as follows: (a) In the S$_0$ state, an increase in conjugation, i.e. lengthening (shortening) of the C=C (C—C) bond, takes place from both ends toward the center

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of the conjugated chain. Both cis-to-trans and trans-to-cis isomerizations in the central part take place in thermal isomerization. (b) In the T_1 state, cis-to-trans isomerization is predominant. The quantum yields of triplet-sensitized isomerization of the central cis (13-cis and 15-cis) isomers were much higher than those of the peripheral cis (7-cis and 9-cis) isomers. The efficient T_1-state isomerization of the central cis isomers was explained in terms of "the triplet-excited region", which is located in the central part of the conjugated chain.[14,15] (c) No isomerization takes place in the S_1 state. Upon direct photoexcitation, intersystem crossing takes place with a quantum yield of $\sim 10^{-3}$, and then isomerization takes place in the T_1 state.

In spinach and algae, β-carotene (a nonpolar symmetric carotenoid) is specifically bound to the PS I and PS II RCs, and lutein, violaxanthin, and neoxanthin (xanthophylls) are bound to the light-harvesting complexes.[16,17] In marine algae such as diatoms, on the other hand, peridinin and fucoxanthin (polar asymmetric carotenoids) play a most important role in the light-harvesting function, and the efficiency of energy transfer to chlorophylls is extremely high.[18] Therefore, we addressed the question, How can the polarity of carotenoids affect the ground-state and the excited-state properties of the conjugated chain? We have chosen β-apo-8′-carotenal as a prototype polar carotenoid and examined the S_0-, S_1-, and T_1-state properties of its isomers. In this paper, we will describe all the spectroscopic results as concisely as possible (the contents of which correspond to those of the series of investigations of β-carotene referred to above) and compare the ground-state and excited-state properties between β-apo-8′-carotenal and β-carotene. [Resonance Raman spectroscopy of all-trans β-apo-8′-carotenal in the S_0, S_1, and T_1 states was reported previously.[19]]

Experimental Section

Isolation of the Isomers by HPLC. All-trans β-apo-8′-carotenal was purchased from Fluka and recrystallized from *n*-hexane. Each isomer was isolated by HPLC from a mixture of isomers which was obtained by iodine-catalyzed photoisomerization of the all-trans isomer in *n*-hexane.[5] HPLC conditions: column, 4-mm i.d. × 250-mm packed with LiChrosorb Si 60, 5 μm (or Daisogel Si sp 60, 5 μm); eluent, 3–7% diethyl ether in *n*-hexane; flow rate, 1.0–1.5 mL/min. (A 8-mm i.d. × 250-mm column was used for preparative purposes.)

NMR and Electronic Absorption Measurements. The NMR spectrum of each isomer was recorded in benzene-d_6 (CEA > 99.97%) solution by the use of a JEOL GX-400, GSX-400, or GSX-600 NMR spectrometer; the values of chemical shifts were determined with reference to H (7.16 ppm) of benzene. The conditions for measurements were as follows. H, H-COSY: pulse sequence, RD–90°–t_1–45°–t_2; digital resolution for the δ_1 and δ_2 axes, 5.85 Hz. H,H-ROESY: pulse sequence, RD–90°–t_1–Δ_1(spin–rock)–t_2; digital resolution for the δ_1 and δ_2 axes, 6.4 Hz. C,H-COLOC: pulse sequence, RD–90°–(Δ_1–$t_{1/2}$)–180°–$t_{1/2}$–Δ_2–decouple for H, RD–(Δ_1–$t_{1/2}$)–180°–$t_{1/2}$–90°–Δ_2–detect for C; digital resolution for the δ_1 (δ_2) axis, 6.8 (18.6) Hz.

The electronic absorption spectrum of each isomer was recorded in *n*-hexane solution on a Shimadzu UV-200 spectrophotometer.

Raman Measurements. The Raman spectrum of each isomer in the S_0 state was recorded for *n*-hexane solution (1×10^{-3} M) at liquid-nitrogen temperature by the use of the 488-nm line (40 mW) of a Lexcel 95 Ar^+ laser and a Jasco TRS-300 Raman spectrometer equipped with a Princeton Instruments IRY-700 detector.

For a set of isomers, for which a large amount of pure sample to make a stable jet stream was readily available, i.e. all-trans, 9-cis, 13-cis, and 13′-cis, both the S_1 and T_1 Raman spectra were extracted from a set of data obtained for *n*-hexane solution (OD_{355} = 8 cm^{-1}; 2×10^{-4} to 1×10^{-3} M) by using the 355-nm and 532-nm, mode-locked (76 MHz) and Q-switched (800 Hz) pulse (<100 ps) trains from a Nd:YAG laser (Quantronix 416). The S_1 Raman spectrum was obtained by subtraction between a pair of spectra with different delay times, i.e. "0 ps" minus 500 ps. The T_1 Raman spectrum was obtained by subtracting a probe-only spectrum from a pump-and-probe spectrum (delay time, 500 ps). The details were described elsewhere.[12,19]

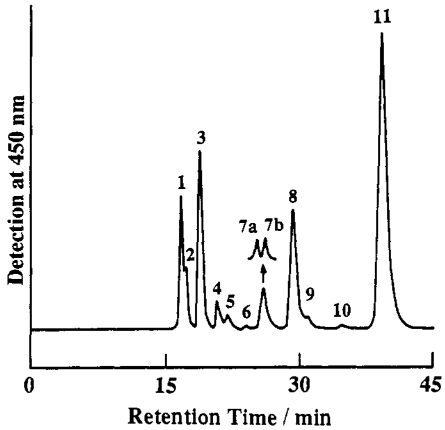

Figure 1. Elution profile of an isomeric mixture of β-apo-8′-carotenal, which was obtained by iodine-catalyzed photoisomerization of the all-trans isomer in *n*-hexane. Assignment of peaks is as follows: peak 1, 13′-cis; peak 2, 15-cis; peak 3, 13-cis, peak 4, 9,13′-cis; peak 7a, 13,13′-cis; peak 7b, 9,13-cis; peak 8, 9-cis; peak 10, 7-cis; peak 11, all-trans.

Table I. Chemical Shifts of the C and H Signals of All-Trans β-Apo-8′-carotenal in Deuteriobenzene (ppm)

C signals				H signals	
8C	138.5	1C	34.5	2H	1.49
8′C	193.6				
		2C	39.8	3H	1.60
9C	136.9				
9′C	137.2	3C	19.7	4H	1.98
10C	131.7	4C	33.3	1Me	1.15
10′C	148.4				
		5C	129.7	5Me	1.82
11C	126.5				
11′C	123.1	6C	138.2	9Me	1.93
				9′Me	1.85
12C	137.5	7C	127.4		
12′C	145.4			13Me	1.86
		1Me	29.2	13′Me	1.71
13C	138.6				
13′C	135.5	5Me	22.1		
14C	132.7	9Me	12.8		
14′C	137.5	9′Me	9.8		
15C	133.2	13Me	12.9		
15′C	129.8	13′Me	12.5		

For the rest of the isomers except for 9,13′-cis, i.e. 7-cis, 15-cis, 9,-13-cis, and 13,13′-cis, S_1 Raman spectra were obtained by using the 527-nm pulses (70 ps, 1 KHz) as the difference spectrum of a high-power spectrum (50 mW) minus a low-power spectrum (5 mW). The above pulses were obtained as the SH from a combination of a CW-ML, Nd:YLF laser (Quantronix 4217) and a Nd:YLF regenerative amplifier (Quantronix 4400). The *n*-hexane solution of each isomer (1×10^{-4} M) was passed through a glass tubing at the linear speed of 13 cm s^{-1}. The T_1 Raman spectrum was obtained for a *n*-hexane solution (1×10^{-3} M). The T_1 species was obtained by triplet sensitization, i.e. the sensitizer, anthracene (10^2 equiv), was excited by the 337-nm pulses from a N_2 laser (NDC JL-1000L), and the T_1 species generated was detected by using the 532-nm pulses from a Nd:YAG laser (Lumonics HY-420), the repetition rate being 10 Hz. The details of the experimental procedure were described elsewhere.[9]

Isomerization Experiments. The relative quantum yields and the primary products of triplet-sensitized isomerization starting from each isomer were determined by HPLC analyses after irradiation of a mixture of an isomer (5×10^{-5} M) and anthracene (5×10^{-3} M) in *n*-hexane with the 337-nm pulses from the N_2 laser. The details of the experimental procedure were described elsewhere.[14]

PPP–SD–CI Calculations. The following relations for the C=O bond were assumed in the calculations of the π-bond order of a model aldehyde,

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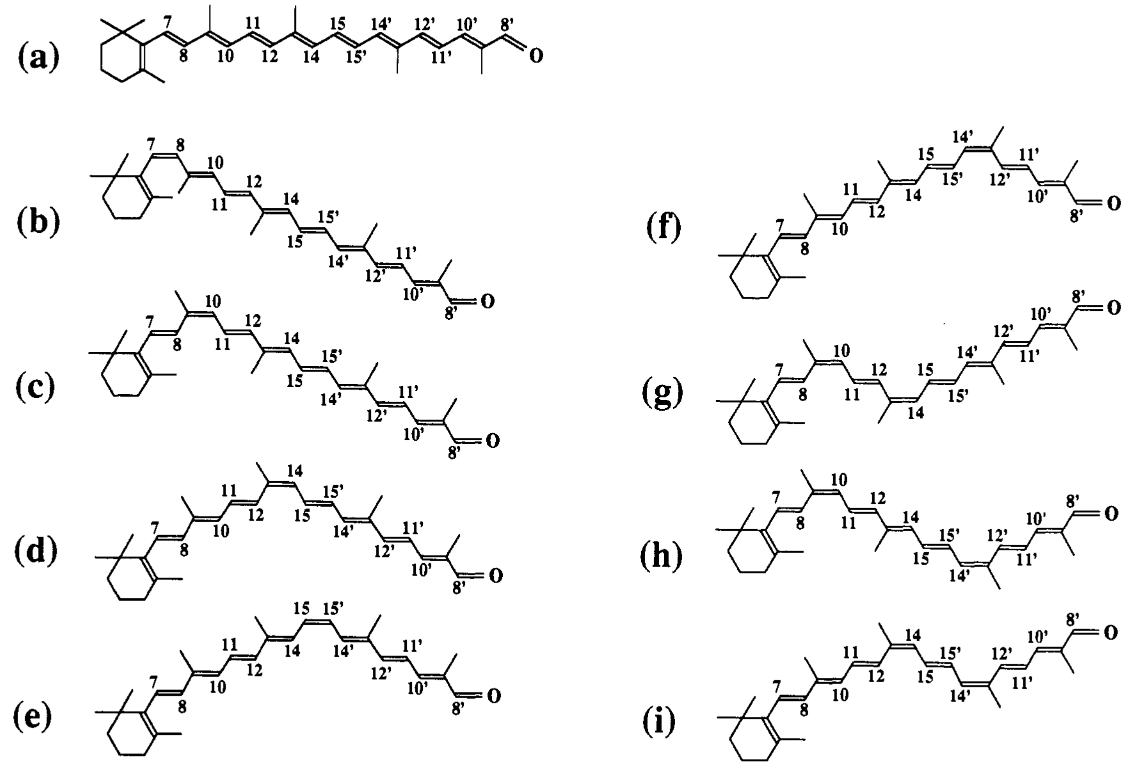

Figure 2. A set of cis–trans isomers of β-apo-8′-carotenal, the configurations of which were determined in the present investigation: (a) all-trans, (b) 7-cis, (c) 9-cis, (d) 13-cis, (e) 15-cis, (f) 13′-cis, (g) 9,13-cis, (h) 9,13′-cis, and (i) 13,13′-cis.

Table II. Chemical Shifts (Isomerization Shifts) of the Olefinic H Signals of Isomeric β-Apo-8′-carotenal in Deuteriobenzene (ppm).

	peak 11 (all-trans)	peak 10 (7-cis)	peak 8 (9-cis)	peak 3 (13-cis)	peak 2 (15-cis)	peak 1 (13′-cis)	peak 7b (9,13-cis)	peak 4 (9,13′-cis)	peak 7a (13,13′-cis)
7H	6.38	5.98 ($\underline{-0.40}$)[a]	6.39 (+0.01)	6.42 (+0.04)		6.40 (+0.02)	6.41 (+0.03)	6.38 (+0.00)	6.38 (+0.00)
8H	6.34	6.27 ($\underline{-0.07}$)	7.08 ($\underline{+0.74}$)	6.36 (+0.02)		6.35 (+0.01)	7.10 ($\underline{+0.76}$)	7.08 ($\underline{+0.74}$)	6.46 ($\underline{+0.12}$)
8′H	9.45	9.47 (+0.02)	9.46 (+0.01)	9.47 (+0.02)	9.44 (–0.01)	9.49 (+0.04)	9.47 (+0.02)	9.49 (+0.04)	9.50 (+0.05)
10H	6.32	6.48 ($\underline{+0.16}$)	6.19 ($\underline{-0.13}$)	6.34 (+0.02)	6.37 (+0.05)	6.32 (+0.00)	6.21 ($\underline{-0.11}$)	6.18 ($\underline{-0.14}$)	6.40 ($\underline{+0.08}$)
10′H	6.52	6.49 (–0.03)	6.51 (–0.01)	6.51 (–0.01)	6.39 ($\underline{-0.13}$)	6.44 ($\underline{-0.08}$)	6.47 (–0.05)	6.47 (–0.05)	6.46 (–0.06)
11H	6.81	6.83 (+0.02)	7.08 ($\underline{+0.27}$)	6.82 (+0.01)	6.87 (+0.06)	6.83 (+0.02)	7.11 ($\underline{+0.30}$)	7.08 ($\underline{+0.27}$)	6.84 (+0.03)
11′H	6.48	6.47 (–0.01)	6.47 (–0.01)	6.47 (–0.01)		6.46 (–0.02)	6.46 (–0.02)	6.46 (–0.02)	6.45 (–0.03)
12H	6.45	6.46 (+0.01)	6.42 (–0.03)	7.01 ($\underline{+0.56}$)	6.54 ($\underline{+0.09}$)	6.49 (+0.04)	6.98 ($\underline{+0.53}$)	6.44 (–0.01)	7.04 ($\underline{+0.59}$)
12′H	6.43	6.47 (+0.04)	6.42 (–0.01)	6.44 (+0.01)	6.46 (+0.03)	7.01 ($\underline{+0.58}$)	6.47 (+0.04)	6.99 ($\underline{+0.56}$)	6.99 ($\underline{+0.56}$)
14H	6.27	6.29 (+0.02)	6.27 (+0.00)	6.09 ($\underline{-0.18}$)	6.86 ($\underline{+0.59}$)	6.38 ($\underline{+0.11}$)	6.05 ($\underline{-0.22}$)	6.35 ($\underline{+0.08}$)	6.19 ($\underline{-0.08}$)
14′H	6.32	6.32 (+0.00)	6.29 (–0.03)	6.34 (+0.02)	6.87 ($\underline{+0.55}$)	6.15 ($\underline{-0.17}$)	6.36 (+0.04)	6.12 ($\underline{-0.20}$)	6.17 ($\underline{-0.15}$)
15H	6.73	6.75 (+0.02)	6.69 (–0.04)	6.98 ($\underline{+0.25}$)	6.52 ($\underline{-0.21}$)	6.63 ($\underline{-0.10}$)	6.99 ($\underline{+0.26}$)	6.57 ($\underline{-0.16}$)	6.88 ($\underline{+0.15}$)
15′H	6.57	6.58 (+0.01)	6.56 (–0.01)	6.51 (–0.06)	6.34 ($\underline{-0.23}$)	6.80 ($\underline{+0.23}$)	6.50 ($\underline{-0.07}$)	6.77 ($\underline{+0.20}$)	6.74 ($\underline{+0.17}$)

[a] Isomerization shifts larger than 0.06 are underlined.

nonadeca-2,4,6,8,10,12,14,16,18-nonaen-1-al[20]:

$$\beta_{rs} = -2.74 + 4.21(r_{rs} - 1.200)$$

$$r_{rs} = 1.424 - 0.216P_{rs}$$

where β_{rs}, r_{rs}, and P_{rs} are the resonance integral, the bond length, and the π-bond order for the C_r–C_s bond. The details are described elsewhere.[14] The threshold value δ of 2 × 10^{-3} eV was used in the selection of CI.

Results and Discussion

HPLC Elution Profile of Isomeric β-Apo-8′-carotenal. Figure 1 shows the elution profile of an isomeric mixture of β-apo-8′-carotenal, which was obtained by iodine-catalyzed photoisomerization of the all-trans isomer in *n*-hexane. A total of 11 peaks were identified by the use of a column which was packed with LiChrosorb Si 60, 5 μm. Peak 7 was split further into two components (7a and 7b) by the use of a column packed with Daisogel Si sp 60, 5 μm. Assignment of the peaks is as follows (*vide infra*): peak 1, 13′-cis; peak 2, 15-cis; peak 3, 13-cis; peak 4, 9,13′-cis; peak 7a, 13,13′-cis; peak 7b, 9,13-cis; peak 8, 9-cis; peak 10, 7-cis; peak 11, all-trans. Peaks 5, 6, and 9 are left to be assigned.

Figure 2 shows a set of cis–trans isomers, the configurations of which have been determined in the present investigation. The isomers elute in the following order: central cis isomers (13′-cis, 15-cis, and 13-cis), di-cis isomers (9,13′-cis, 13,13′-cis, and 9,13-cis), peripheral cis isomers (9-cis and 7-cis), and finally, the all-trans isomer. The results suggest that the geometrical factor, i.e. the intermolecular interaction between each bent or stretched, conjugated backbone and a microscopically flat surface of silica gel, determines the retention time. The strongest interaction is expected for the all-trans isomer and next for the peripheral cis isomers; the weakest interactions are expected for the central cis isomers. [The trend parallels not to the case of β-carotene[5,6] but to the case of neurosporene.[21]]

Structures of the Isomers as Determined by NMR Spectroscopy. Table I lists the values of chemical shifts for the C and H signals (^{13}C and ^{1}H are abbreviated as C and H, respectively, in this

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paper) of all-trans β-apo-8′-carotenal in benzene. (The chemical shifts for the olefinic H signals are listed in Table II.) The signals were assigned not empirically but completely logically by the use of COSY (correlation spectroscopy), ROESY [rotating frame nuclear Overhauser effect (NOE) spectroscopy], and COLOC (correlation spectroscopy via long-range coupling) spectra. The all-trans configuration was confirmed by a set of H,H-NOE peaks (data not shown) correlating 1Me, 7H, 9Me, 11H, 13Me, 15H, 14′H, and 12′H, and those correlating 12H, 14H, 15′H, 13′Me, 11′H, and 9′Me (see Figure 2). The chemical shifts of the olefinic carbons show the following characteristics: (1) Unmethylated carbons in the conjugated chain in which severe steric interaction between the methyl groups and the attached protons is expected, i.e. 7C, 11C, and 11′C, show low values of chemical shifts (123–127 ppm); the electrons must be pushed from the hydrogen to the carbon atoms, due to the above van der Waals interaction between the hydrogen atoms, and as a result, the electron density on the above carbon atoms must be increased. (2) 15C and 15′C of which attached hydrogens interact with the 13Me and 13′Me hydrogens also show low values of chemical shifts (130–133 ppm). (3) Among the rest of the olefinic carbons, 8′C, 10′C, and 12′C (194, 148, and 145 ppm) show much higher chemical shifts than 8C, 10C, and 12C (139, 132, and 138 ppm); electrons seem to be withdrawn by oxygen from carbon atoms in the carbonyl end.

Table II lists the chemical shifts of the olefinic H signals for the isomers named peaks 11, 10, 8, 3, 2, 1, 7b, 4, and 7a. Shown in parentheses are the values of "isomerization shifts", i.e. changes in the chemical shifts of the olefinic H signals in reference to those of the all-trans isomer. Each cis configuration was identified by the use a general rule of the "isomerization shifts": when a cis bend is introduced into a stretched conjugated chain, high-field shifts (hfs) of the olefinic H signals take place at the convex side of the cis bend, while low-field shifts (lfs) take place at the concave side.

First of all, peak 11 turned out to be the all-trans isomer (*vide supra*). In comparison with the case of the all-trans isomer, peak 10 shows the hfs of the 7H and 8H signals and the lfs of the 10H signal; therefore, it is assigned to the 7-cis isomer (compare Table II and Figure 2). Peak 8 shows the hfs of the 10H signal and the lfs of the 8H and 11H signals; thus it is assigned to the 9-cis isomer. Peak 3 shows the hfs of the 14H signal and the lfs of the 12H and 15H signals; it is assigned to the 13-cis isomer. Peak 2 shows the hfs of the 15H and 15′H signals and the lfs of the 14H and 14′H signals, and it is assigned to the 15-cis isomer. Peak 1 shows the hfs of the 14′H signal and the lfs of the 12′H and 15′H signals and is assigned to the 13′-cis isomer.

Peak 7b shows the characteristics of both the 9-cis configuration (the hfs of 10H and the lfs of 8H and 11H) and the 13-cis configuration (the hfs of 14H and the lfs of 12H and 15H); therefore, it is assigned to the 9,13-di-cis isomer. The 9,13-cis configuration was confirmed by a set of H,H-NOE peaks correlating 7H, 9Me, 10H, and 12H and those correlating 11H, 13Me, 14H, and 15′H (data not shown). Peak 4 shows the characteristics of both the 9-cis configuration (the hfs of 10H and the lfs of 8H and 11H) and the 13′-cis configuration (the hfs of 14′H and the lfs of 12′H and 15′H), and it is assigned to the 9,13′-di-cis isomer. Peak 7a shows the characteristics of both the 13-cis configuration (the hfs of 14H and the lfs of 12H and 15H) and those of the 13′-cis configuration (the hfs of 14′H and the lfs of 12′H and 15′H); thus, it is assigned to the 13,13′-di-cis isomer. This configuration was confirmed by H,H-NOE peaks correlating 12H and 15H (12′H and 15′H) and those correlating 11H, 13Me, 14H, and 15′H (11′H, 13Me, 14′H, and 15H).

In comparison with the case of isomeric β-carotene,[7] the effect of a cis bend on the chemical shifts tends to extend further into the rest of the all-trans part(s). Because of the polar group at one end, introduction of a cis bend may cause redistribution of the electrons all over the conjugated chain.

Table III. Vicinal Coupling Constants of the Olefinic H's for Isomeric β-Apo-8′-carotenal in Deuteriobenzene (Hz)

	all-trans	7-cis	9-cis	13-cis	15-cis	13′-cis	9,13-cis	9,13′-cis	13,13′-cis
7H=8H	16	13	16	17		16	16	16	16
10H—11H	12	12	11	12	11	12	12	12	13
10′H—11′H	12	11	11	12	11	12	12	12	13
11H=12H	14	15	15	15	15	15	15	15	15
11′H=12′H	14	15	15	16	16	15	15	15	15
14H—15H	12	13	11	12	12	12	12	11	12
14′H—15′H	12	13	11	12	12	12	12	12	11
15H=15′H	15	14	14	13	12	14	15	14	14

Table IV. Electronic Absorption Bands of Isomeric β-Apo-8′-carotenal in *n*-Hexane (nm)

	$^1A_g^- \rightarrow {}^1A_g^+$ absorption		$^1A_g^- \rightarrow {}^1B_u^+$ absorption			$^1A_g^- \rightarrow {}^1A_g^+ / {}^1A_g^- \rightarrow {}^1B_u^+$ intensity ratio[a]
	(0–1)	(0–0)	(0–2)	(0–1)	(0–0)	
all-trans		328	429	453	480	0.01
7-cis		331	427	451	480 (0)[b]	0.03
9-cis		328	427	450	477 (–3)	0.02
13-cis	317	330	425	448	474 (–6)	0.41
15-cis	316	328	425	448	475 (–5)	0.48
13′-cis	318	331	423	445	470 (–10)	0.37
9,13-cis	312	327	422	443	468 (–12)	0.09
9,13′-cis	312	328	420	443	469 (–11)	0.08
13,13′-cis		332	420	438	464 (–16)	0.04

[a] Peak intensity at the maximum. [b] Shifts in reference to the absorption in the all-trans isomer (isomerization shift).

Table III lists the H,H-vicinal coupling constants through the C=C and C—C bonds in the conjugated chain. In the case of the all-trans isomer, the coupling constants on both sides are symmetric [J(10H—11H) = J(10′H—11′H), etc.], despite the asymmetry of the molecular structure and resultant chemical shifts. The coupling constants of the central double bonds [J(11H=12H), J(15H=15′H), and J(11′H=12′H)] are smaller than that of the terminal double bond [J(7H=8H)]; this is due to the fact that the effect of conjugation is increased in the central part (see below).

In the case of all-trans β-carotene,[7] the vicinal coupling constant of the C=C bond decreases, while that of the C—C bond increases systematically from both ends toward the center of the extended conjugated chain; the results were interpreted as that the effect of conjugation is increased in the central part of the chain, and as a result, the C=C (C—C) bond is elongated (shortened).[7] This trend is not so clearly seen in all-trans β-apo-8′-carotenal.

In the case of the cis isomers of the present carotenoid, the order of the vicinal coupling constant for the double bond, J(7H=8H) > J(11H=12H) > J(15H=15′H), is seen for 9-cis, 13-cis, 13′-cis, 9,13′-cis, and 13,13′-cis. The trend found in all-trans and isomeric β-carotene may still remain in these isomers. As for the vicinal coupling constants for the single bond, J(10H—11H) = J(14H—15H) [J(10′H—11′H) = J(14H—15H)] is seen for all-trans, 9-cis, 13-cis, 13′-cis, and 9,13-cis. The lengths of the above single bonds may be similar in this carotenoid.

The vicinal coupling constant for the central cis double bond [J(cis 15H=15′H)] is smaller than that for the peripheral cis double bond [J(cis 7H=8H)]. This can also be ascribed to the increased conjugation in the central part. The same trend was seen in the case of β-carotene.[7]

Electronic Absorptions of the Isomers. Table IV lists the wavelengths of the strongly optically-allowed $^1A_g^- \rightarrow {}^1B_u^+$ absorption and of the $^1A_g^- \rightarrow {}^1A_g^+$ absorption called the "cis-peak"; it lists also the intensity ratio of the absorptions, $^1A_g^- \rightarrow {}^1A_g^+ / {}^1A_g^- \rightarrow {}^1B_u^+$. The "isomerization shift", i.e. the shift of the $^1A_g^- \rightarrow {}^1B_u^+$ (0–0) absorption in each cis isomer in reference to that of the all-trans isomer is in the order 7-cis < 9-cis < 15-cis

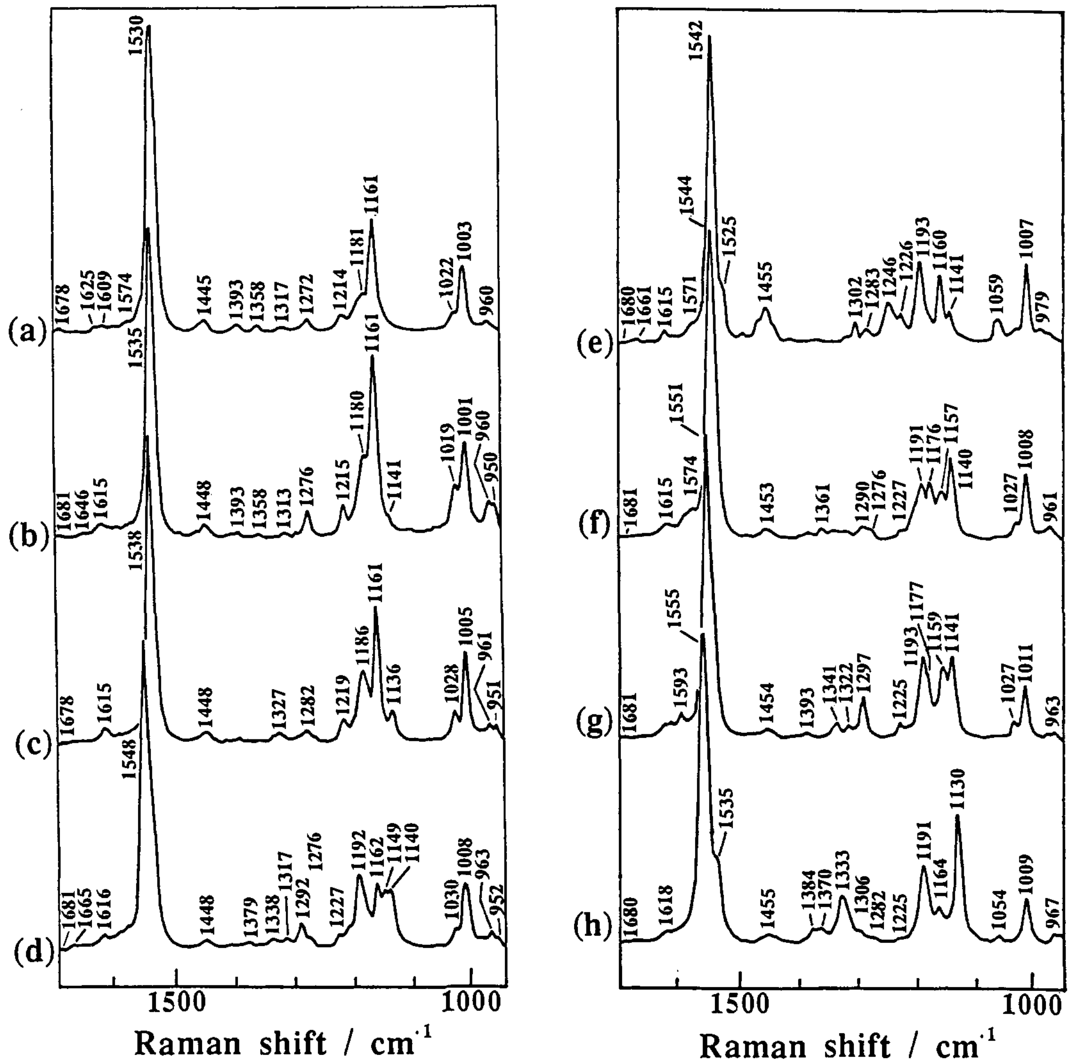

Figure 3. Raman spectra of the (S_0-state) isomers of β-apo-8′-carotenal: (a) all-trans, (b) 7-cis, (c) 9-cis, (d) 13-cis, (e) 15-cis, (f) 13′-cis, (g) 9,13-cis, and (h) 13,13′-cis.

Table V. Frequencies and Intensities of Stretching Raman Lines in Isomeric β-Apo-8′-carotenal (cm^{-1})

	all-trans	7-cis	9-cis	13-cis	15-cis	13′-cis	9,13-cis	13,13′-cis
C=O stretching	1678vw	1681vw	1678vw	1681vw	1680vw	1681vw	1681vw	1680vw
C=C stretching	1530s	1535s	1538s	1548s	1542s	1544s	1551s	1555s
C—C stretchings								
12—13, 12′—13′	1181m	1180m	1186m	1192s[a]	1193s	1176m 1191m	1193m	1191s
14—15, 14′—15′	1161s	1161s	1161s	1162m	1141w	1157m	1159m	1164w
10—11, 10′—11′	nd	1141vw	1136w	1140m 1149w	1160s	1140s	1141m	1130s

[a] Key Raman lines of configurations are underlined.

< 13-cis < 13′-cis, for the set of mono-cis isomers. The "isomerization shifts" for the di-cis isomers are higher.

The transition moment of the $^1A_g^- \rightarrow {}^1B_u^+$ transition is along the long axis and that of the $^1A_g^- \rightarrow {}^1A_g^+$ transition is along the short axis of each isomer; therefore, the intensity ratio should change reflecting each cis–trans configuration. The ratio is in the order 15-cis > 13-cis > 13′-cis ≫ 9,13-cis ≥ 9,13′-cis, in accord with each configuration (see Figure 2); the ratio is much lower for the 13,13′-cis, the peripheral cis (7-cis and 9-cis), and the all-trans isomers.

Similar trends in the isomerization shift and in the intensity ratio were found for isomeric β-carotene.[22]

Raman Spectra of the (S_0-State) Isomers and the Key Raman Lines of Cis–Trans Configurations. Figure 3 shows the S_0 Raman spectra of the above set of cis–trans isomers (except for 9,13′-cis). Table V lists a set of empirical assignments of the C=O, C=C, and C—C stretching Raman lines. The Raman line with the highest frequency (1681–1678 cm^{-1}) is assigned to the stretching mode of the terminal carbonyl group. A strong infrared absorption at 1670 cm^{-1} of crystalline all-trans β-apo-8′-carotenal (data not shown) supports this assignment. [The C=O stretching frequency must be lowered in the crystal due to a hydrogen bond-like interaction with the 8′H of the neighboring molecule; note that its NMR signal is shifted to the lower field considerably (see Table II), a fact which indicates the removal of electrons from 8′H (positive polarization on 8′H).]

The strongest Raman line can be assigned to the C=C stretching vibration, in which all the C=C bonds stretch inphase and give rise to the high Raman intensity. (Additional weak Raman lines on both sides can be ascribed to other C=C stretching

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[22] Koyama, Y.; Takii, T.; Saiki, K.; Tsukida, K. *Photobiochem. Photobiophys.* **1983**, *5*, 139–150.

Table VI. UT, UC, MT, and MC Modes Characteristic of Unmethylated Trans, Unmethylated Cis, Methylated Trans, and Methylated Cis Structures

	all-trans	7-cis	9-cis	13-cis	15-cis	13′-cis	9,13-cis	13,13′-cis	all-trans
	(S$_0$ state)								(T$_1$ state)
7=8		1276m (UC)[a]	1282w (UT)						
7′=8′									
11=12			1282w (UT)						
	1272w (UT)			1292m (UT)	1283w (UT)	1276w (UT)	1297s (UT)	1282w (UT)	1250m (UT)
11′=12′									
15=15′					1246m (UC)				1236 (UT)
9=10			1219m (MC)				1225w (MC)		
9′=10′									
13=4				1227w (MC)					
	1214w (MT)	1215m (MT)			1226w (MT)			1225w (MC)	1198w (MT)
13′=14′						1227w (MC)	1225w (MC)		
	(S$_1$ state)								
7=8									
		1247s (UC)							
7′=8′									
11=12	1285s (UT)								
			1282m (UT)	1282s (UT)	1268m (UT)	1282vs (UT)	1283m (UT)	1286s (UT)	
11′=12′									
15=15′	1260s (UT)		1260s (UT)	1260m (UT)	1248s (UC)		1241m (UT)		
9=10			1200m (MC)						
9′=10′									
13=14				1229m (MC)					
	1209m (MT)	1208m (MT)			1210m (MT)			1235w (MC)	
13′=14′						1206m (MC)			

[a] Key Raman lines of configurations are underlined.

modes with various phase differences of vibrations between adjacent C═C units.) The inphase C═C stretching Raman line shifts to the higher frequencies in the order all-trans (0) < 7-cis (5) < 9-cis (8) < 15-cis (12) < 13′-cis (14) < 13-cis (18) < 9,13-cis (21) < 13,13′-cis (25); shown in parentheses are the isomerization shifts. The order parallels that in the case of β-carotene,[8,22] i.e. all-trans (0) < 7-cis (1) < 9-cis (5) < 15-cis (10) < 13-cis (13) < 9,13-cis (14) < 13,13′-cis (25). However, the isomerization shifts for β-apo-8′-carotenal tend to be slightly higher than those for β-carotene.

The Raman lines in the regions of 1455–1445 cm^{-1} and of 1393–1384 cm^{-1} can be assigned to the methyl asymmetric and symmetric deformations, respectively. Raman lines in the 1360–1200-cm^{-1} region are mainly associated with the C—H in-plane (ip) bending vibrations. In particular, a set of vibrational modes which are characteristic of the unmethylated trans, unmethylated cis, methylated trans, and methylated cis structures and called the "UT", "UC", "MT", and "MC" modes have been found in the cases of retinal,[23] its homologs,[24] and β-carotene.[8] (a) The UT and UC modes appear in the region of 1290–1240 cm^{-1}. The UT mode is an a_g-type vibration associated with the trans C—CH═CH—C structure (local symmetry C_{2h}), in which a pair of C—H ip bendings and the C═C stretching are coupled with one another. The UC mode, on the other hand, is an a_1-type vibration associated with the cis C—CH═CH—C structure (local symmetry C_{2v}), in which a pair of the C—H ip bendings and the C═C stretching are coupled with one another. The UT and UC modes for the central C14—C15H═C15H—C14 structure appear in the lower-frequency region. (b) The MT and MC modes appear in the region of 1230–1210 cm^{-1}. The MT and MC modes take place within the trans and cis C—C*-(CH_3)═CH—C structures and they consist of the C—C* stretching, the methyl ip locking, and the C—H ip bending. As for the intensities of those modes, it has been generally found that those vibrational modes which take place either in the central part of an extended chain or around the cis bend are most strongly resonance-enhanced. Table VI lists a set of those characteristic modes which are assigned on the basis of the above empirical rule. In particular, the Raman line of the 7-cis isomer at 1276 cm^{-1} and that of the 15-cis isomer at 1246 cm^{-1} are regarded as the key Raman lines of those configurations.

Raman lines in the 1200–1130-cm^{-1} region can be mainly ascribed to the C–C stretchings, the assignment of which is listed in Table V. The relative intensity of the C10–C11 (C10′–C11′) stretching (~1140 cm^{-1}) *vs* the C14–C15 (C14′–C15′) stretching (~1160 cm^{-1}) can be used to differentiate a central cis (13-cis or 13′-cis) configuration from a peripheral cis (9-cis or 7-cis) and the all-trans configuration (see also Figure 3). The C10–C11 (C10′–C11′) stretching Raman line is not seen for the all-trans isomer, it is weakly seen for the 7-cis isomer, and it gets stronger for the 9-cis isomer. Its intensity becomes comparable to that of the C14–C15 (C14′–C15′) stretching for the 13-cis isomer, and the intensity becomes even higher for the 13′-cis isomer. Finally, the relative intensity is completely reversed for the 13,13′-cis isomer. [In the case of the 15-cis isomer of β-carotene, the calculations of normal vibrations revealed that the frequency of the C14–C15 (C14′–C15′) stretching and that of the C10–C11 (C10′–C11′) stretching are reversed.]

The C12–C13 (C12′–C13′) stretching shows a similar trend: its intensity increases in the order all-trans < 7-cis < 9-cis. The intensity becomes much higher for the 13-cis, 15-cis, and 13′-cis isomers (the frequency also becomes slightly higher). In the case of the 13,13′-cis isomer, the intensity of the particular mode becomes much higher than that of the C14–C15 (C14′–C15′) stretching (1164 cm^{-1}). The Raman line around 1192 cm^{-1} can be regarded as a key Raman line of a central cis (13-cis, 15-cis, or 13′-cis) configuration.

Parallel results were obtained for isomeric β-carotene,[8] supporting the above interpretations. The above empirical assignment

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(23) Koyama, Y.; Mukai, Y.; Umemura, J.; Ito, M.; Tsukida, K. *J. Raman Spectrosc.* **1984**, *15*, 300–307.

(24) Mukai, Y.; Koyama, Y.; Ito, M.; Tsukida, K. *J. Raman Spectrosc.* **1986**, *17*, 387–396.

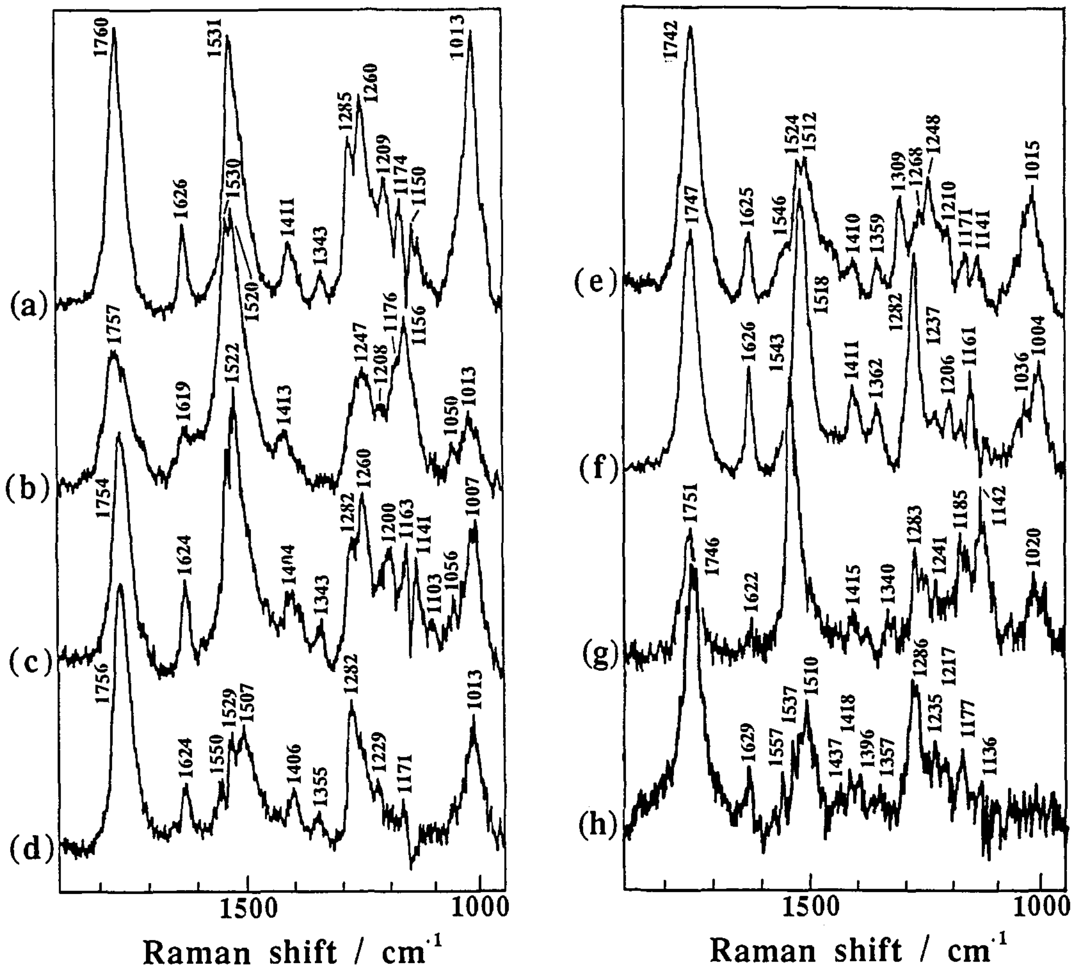

Figure 4. Raman spectra of S_1 species generated from isomeric β-apo-8′-carotenal: (a) all-trans, (b) 7-cis, (c) 9-cis, (d) 13-cis, (e) 15-cis, (f) 13′-cis, (g) 9,13-cis, and (h) 13,13′-cis.

and interpretation of the Raman lines seem to be plausible, but they need to be confirmed by calculations of normal vibrations using a set of force constants determined for the Raman data of undeuterated and variously deuterated homologues.

Configurations of S_1 Species Generated from the Isomers as Probed by Raman Spectroscopy. Figure 4 shows the Raman spectra of S_1 species which were recorded by a two-color (all-trans, 9-cis, 13-cis, and 13′-cis) or one-color (7-cis, 15-cis, 9,13-cis, and 13,13′-cis) method using picosecond pulses. The strong Raman line with the highest frequency (1760–1740 cm^{-1}) can be assigned to the C=C stretching vibration in which all the C=C bonds stretch inphase (an a_g-type vibration). As in the case of β-carotene,[11-13,25-27] its abnormally high frequency is ascribed to a vibronic coupling with the S_0 ($^1A_g^-$) state. Thus, the S_1 state probed by transient Raman spectroscopy is the $2^1A_g^-$ state. Here, the S_1 state as the $2^1A_g^-$ state is shown for all the sets of isomers. No systematic changes in frequency are found among the isomers. But, both the highest frequency in the $2^1A_g^-$ (S_1) state and the lowest frequency in the $^1A_g^-$ (S_0) state are found for the all-trans isomer, a fact which indicates that the vibronic coupling is the strongest in this particular isomer.

The weak Raman line around 1626 cm^{-1} is assigned to the C=O stretching vibration. The shift of this mode upon singlet excitation (~55 cm^{-1}) indicates a large decrease in the bond order, the amount of which looks similar from one isomer to another. The strong or medium Raman line in the 1530–1520-cm^{-1} region is ascribed to the C=C stretching Raman line which is not vibronically coupled with the S_0 state (non a_g-type mode). A pair of weak Raman lines around 1410 and 1360 cm^{-1} can be assigned to the asymmetric and symmetric methyl deformations; the frequencies of the particular modes are lowered upon singlet excitation.

A group of Raman lines in the 1300–1100-cm^{-1} region can be ascribed to coupled vibrations consisting of the C—H ip bendings, the C—C stretchings, and the C=C stretchings. The spectral pattern in this region is expected to be very sensitive to the cis–trans configuration, and each isomer shows its unique spectral pattern. The observation indicates that the S_1-state structure is different from one isomer to another. Table VI shows also a set of empirical assignments for the UT, UC, MT, and MC modes in the S_1 state. The assignment based on the general rule of Raman intensity mentioned above strongly suggests that essentially the same planar cis or trans configuration as that in the S_0 state is conserved for each isomer, even after excitation to the S_1 state.

Configurations of T_1 Species Generated from the Isomers as Probed by Raman Spectroscopy. Figure 5 shows the Raman spectra of T_1 species which were generated from the set of isomers either by direct photoexcitation (all-trans, 9-cis, 13-cis, and 13′-cis) or by triplet-sensitized excitation (7-cis, 15-cis, 9,13-cis, and 13,13′-cis). In the case of direct excitation, the T_1 species are generated through intersystem crossing (ISC). Therefore, it is generally expected that each T_1 species with the initial S_0-state configuration is generated immediately after ISC, if there were no isomerization in the S_1 state (*vide supra*); the resultant T_1 species may isomerize into another isomer as in the case of β-carotene. In the case of sensitized excitation, on the other

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(25) Noguchi, T.; Kolaczkowski, S.; Arbour, C.; Aramaki, S.; Atkinson, G. H.; Hayashi, H.; Tasumi, M. *Photochem. Photobiol.* **1989**, *50*, 603–609.

(26) Noguchi, T.; Hayashi, H.; Tasumi, M.; Atkinson, G. H. *Chem. Phys. Lett.* **1990**, *175*, 163–169.

(27) Noguchi, T.; Hayashi, H.; Tasumi, M.; Atkinson, G. H. *J. Phys. Chem.* **1991**, *95*, 3167–3171.

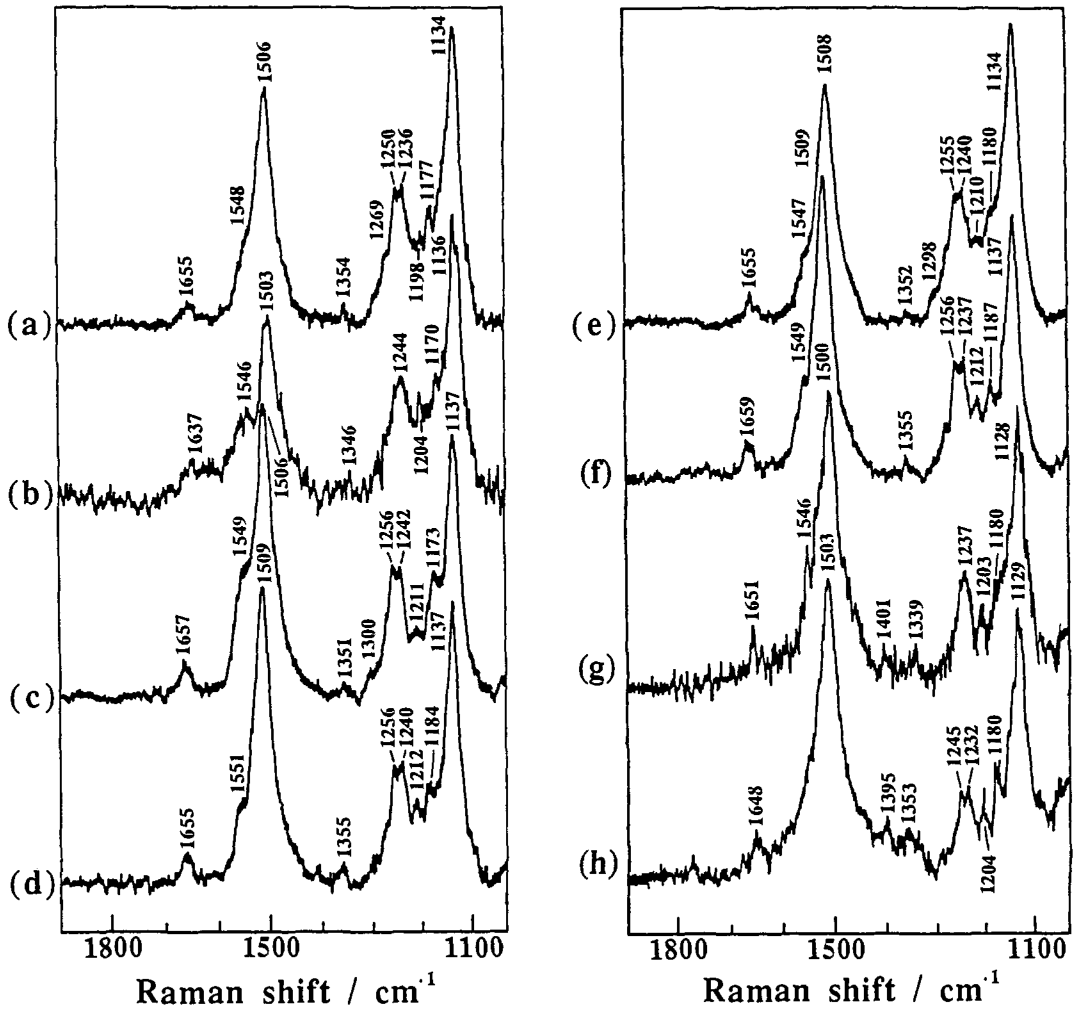

Figure 5. Raman spectra of T_1 species generated from isomeric β-apo-8′-carotenal: (a) all-trans, (b) 7-cis, (c) 9-cis, (d) 13-cis, (e) 15-cis, (f) 13′-cis, (g) 9,13-cis, and (h) 13,13′-cis.

hand, each T_1 species is generated through collision with the sensitizer in the T_1 excited state and subsequent triplet energy transfer. Each cis or trans T_1 species is expected to be generated, in this case also, immediately after energy transfer, but the collision takes place at random. We hoped to detect a unique Raman spectrum of each T_1 species especially in direct photoexcitation, in which all the carotenoid molecules are synchronously excited by photons. However, all the T_1 Raman spectra turned out to be identical, within the limit of experimental error. [The possibility that small differences are overlooked because of the low S/N ratio cannot be completely excluded, at this stage, especially in the case of the 9,13-di-cis isomer (*vide infra*).] However, on the basis of the results of triplet-sensitized isomerization, i.e. all the cis isomers isomerize efficiently into the all-trans isomer (*vide infra*), we ascribe all the spectra obtained for different isomers to the all-trans T_1 species.

The Raman lines of the all-trans T_1 (Figure 5a) characterize its structures as follows: The Raman line at 1655 cm^{-1} can be assigned to the C=O stretching. The frequency of this mode is in the order S_0 (1678 cm^{-1}) > T_1 (1657 cm^{-1}) > S_1 (1626 cm^{-1}), the shift upon triplet (singlet) excitation being 21 (52) cm^{-1}. The results indicate that the effect of excitation reaches to the terminal carbonyl group less effectively in the T_1 state than in the S_1 state (*vide supra*).

The strong Raman line at 1506 cm^{-1} can be ascribed to the C=C stretching mode in which all the C=C bonds stretch inphase. The frequency of the particular mode is in the order S_1 vibronically coupled (1760 cm^{-1}) > S_0 vibronically coupled (1530 cm^{-1}) > T_1 not vibronically coupled (1506 cm^{-1}). Because of the vibronic coupling between the S_1 and S_0 states, the frequency of the particular mode is pushed to the higher (lower) in the S_1 (S_0) state. However, the C=C stretching frequency in the T_1 state is much lower than that in the S_0 state. Since the central carbon–carbon stretching vibrations are expected to be most strongly resonance-enhanced, the result suggests the presence of a large decrease in the bond order in the central part.

The Raman lines in the 1300–1200-cm^{-1} region can be assigned to the UT and MT modes as listed in Table VI. Their frequencies are slightly lower than those in the S_0 state. The Raman lines at 1177 and 1134 cm^{-1} are ascribable to the C–C stretchings. Their frequencies are comparable to those of the S_0-state C–C stretchings. Since the spectral pattern in this region should be very sensitive to the difference in the cis–trans configuration, the same spectral patterns seen for all the T_1 species generated from the set of isomers strongly support the idea that their configurations are identical (the all-trans T_1).

The T_1-state "C=C" and "C—C" stretching frequencies of the present carotenoid are similar to those of β-carotene.[9] The fact suggests that the bond orders in the central part of the conjugated chain are similar between the two carotenoids.

Triplet-Sensitized Isomerization Starting from Each Isomer. The HPLC analyses of triplet-sensitized isomerization starting from the set of cis–trans isomers of β-apo-8′-carotenal are summarized as follows. (1) Relative quantum yields (normalized to that of the 13-cis isomer, which showed the maximum value), which are defined as the decrease in the starting isomer per triplet species generated, were as follows: all-trans, 0.00; 7-cis, 0.40; 9-cis, 0.87; 13-cis, 1.00; 15-cis, 0.34; 13′-cis, 0.33; 9,13-cis, 0.42; 13,13′-cis, 0.28. (2) The major product of isomerization was the all-trans isomer in all the mono-cis isomers. (Both the all-trans and 9-cis isomers were generated from the 9,13-di-cis isomer.) (3) The stationary-state composition (averaged for all the different starting isomers except for 7-cis, in which the stationary state could not be reached under the present experimental conditions) was as follows: all-trans, 85%; 9-cis, 7%; 13-cis, 2%; 13′-cis, 4%; the rest of the isomers, negligible. Although the absolute quantum yields have not been determined yet, the above preliminary results support the idea that the T_1-state isomerization from each cis to all-trans is very efficient and that the T_1 species detected by transient Raman spectroscopy is the all-trans T_1, the result of the T_1-state isomerization (*vide supra*).

In the case of β-carotene,[9,14] the results of triplet-sensitized isomerization can be summarizes as follows. (1) The quantum yields of triplet-sensitized isomerization were as follows: all-trans, 0.04 (0.04); 7-cis, 0.12 (0.12); 9-cis, 0.15 (0.15); 13-cis, 0.87 (0.89); 15-cis, 0.98 (1.00). (Shown in parentheses are relative quantum yields normalized to that of the 15-cis isomer, showing the maximum value.) (2) The major product of isomerization was the all-trans isomer in all the above starting isomers. (3) The stationary-state composition was as follows: all-trans, 85%; 9-cis, 10%; 13-cis, 4%; 15-cis, 1%.

A large difference between the two carotenoids is seen in the relative quantum yields (the major products of isomerization are the same and the stationary-state compositions are similar to one another). For the set of mono-cis and the all-trans isomers, the relative quantum yield is in the order 15-cis > 13-cis ≫ 9-cis > 7-cis ≫ all-trans for β-carotene, while it is in the order 13-cis > 9-cis > 7-cis > 15-cis ≑ 13′-cis ≫ all-trans for β-apo-8′-carotenal. The above orders and the discrete changes in the quantum yields explain nicely the results of transient Raman spectroscopy of T_1 species, i.e. the all-trans, 7-cis, and 9-cis T_1 species were detected in the case of β-carotene,[9] while only the all-trans T_1 was detected in the case of the present carotenoid. The above order of the quantum yield in β-carotene suggests that "the triplet-excited region" is located in the center of the conjugated chain, while the order in β-apo-8′-carotenal suggests that "the triplet-excited region" is shifted from the center of the conjugated chain toward the β-ionone ring.

PPP–SD-CI Calculations of the Bond Order. Figure 6a shows the result of the PPP–SD-CI calculation of the π-bond order for a model compound of β-apo-8′-carotenal (nonadecanonaen-1-al), while Figure 6b shows the result for a model compound of β-carotene (docosaundecaene)[14] for comparison. Main differences in the S_0- and T_1-state properties of the two carotenoids can be summarized as follows. (a) S_0-state properties (open circles): In the case of β-apo-8′-carotenal, the C15═C15′ bond is not at the center of the shortened conjugated chain; this may be the reason why the effect of conjugation is not so clearly seen in the set of vicinal coupling constants. In β-carotene, the C15═C15′ bond is at the center of the symmetric and longer conjugated chain; the effect of conjugation may be larger and easier to be detected. Further, in the case of β-apo-8′-carotenal, the oxygen atom at one end seems to cause a decrease in the bond order of neighboring C═C bonds. The result parallels the observation of higher chemical shifts (low-field shifts) of the relevant carbon atoms, reflecting removal of electrons.

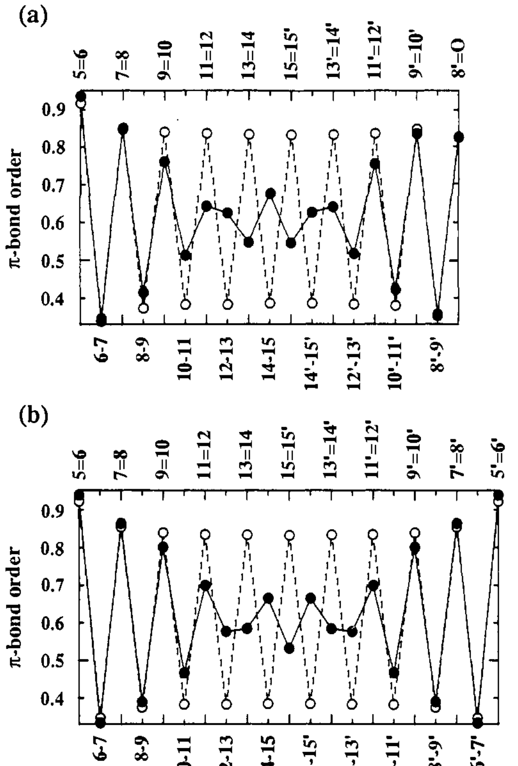

Figure 6. π-Bond orders in the S_0 (○) and T_1 (●) states for each carbon–carbon bond of the model polyenes of (a) β-apo-8′-carotenal and (b) β-carotene, i.e. nonadeca-2,4,6,8,10,12,14,16,18-nonaen-1-al and docosa-1,3,5,7,9,11,13,15,17,19,21-undecaene.[14] The central part, where large changes in the bond order take place, is called "the triplet-excited region".

(b) T_1-state properties (closed circles): In both carotenoids, large changes in the π-bond order take place in the central part of the entire conjugated chain, which we call "the triplet-excited region".[14,15] However, the alternating changes in the bond order seen in the S_0 state are well conserved at both ends. In β-apo-8′-carotenal, the triplet-excited region tends to be more compressed within the shorter conjugated chain, and its effect reaches more readily toward both ends (compare the bond order of the C9═C10 bond, for example). The effect is well reflected in the frequency of the C═O stretching in the T_1 state (*vide supra*). Since the barrier against isomerization is expected to be decreased when the π-bond order is decreased, the general trend of more efficient cis-to-trans isomerization in the case of β-apo-8′-carotenal than in the case of β-carotene is well explained. However, the calculation predicts that the 13-cis and 15-cis isomers should show similar quantum yields of isomerization. Thus, further shift of "the triplet-excited region" toward the β-ionone ring, which has been suggested by the observation of quantum yields (*vide supra*), cannot be explained by the present PPP–SD-CI calculations.

Conclusions. Differences in the S_0-, S_1-, and T_1-state properties between β-apo-8′-carotenal and β-carotene stem from the differences in the chemical structure. The conjugated chain of β-apo-8′-carotenal consists of nine C═C and one C═O bonds, while that of β-carotene consists of eleven C═C bonds. Thus, β-apo-8′-carotenal is characterized by the shorter conjugated chain and by the presence of the carbonyl group at one end (or by asymmetry). (A) Similarities between the pair of carotenoids are mainly ascribable to the intrinsic properties of the conjugated chain: (1) Configurational dependence of the chemical shifts of the olefinic H's and that of the electronic absorptions were similar to one another for the pair of carotenoids. (2) The frequencies of the C═C and C—C stretching Raman lines, which reflect the bond orders in the S_0, S_1, and T_1 states, are also similar. (3) The key Raman lines of each cis–trans configuration in the S_0 state are essentially the same between the two carotenoids except for the effect of asymmetry (i.e. splitting of Raman lines) seen in the present carotenoid. (4) In both carotenoids, the S_1 state probed by transient Raman spectroscopy is the $2^1A_g^-$ state, and no indication of isomerization was seen in the S_1 state. (5) Efficient cis-to-trans isomerization takes place in the T_1 state for both carotenoids.

(B) Differences between the pair of carotenoids are ascribable to the effect of the terminal group and/or to the shortened conjugated chain in β-apo-8′-carotenal. (1) The effect of the terminal oxygen is seen in the higher values of chemical shifts of carbons on the carbonyl side. Systematic change in the vicinal coupling constants from both ends toward the center, which reflects increased conjugation in the central part, is seen not in the case of β-apo-8′-carotenal but in the case of β-carotene. (2) A large difference has been found between the carotenoids in the order of the quantum yield of triplet-sensitized isomerization; the results suggest that "the triplet-excited region" is located in the center of the conjugated chain in the case of β-carotene and that it is shifted toward the β-ionone ring in the case of β-apo-8′-carotenal. (3) A difference corresponding to the above is found in the T_1 species which was detected by transient Raman spectroscopy; the all-trans T_1 alone was detected in β-apo-8′-carotenal, while the all-trans, 7-cis, and 9-cis T_1 species were detected in β-carotene.

Thus, differences in the S_0- and T_1-state properties have been found between β-apo-8′-carotenal and β-carotene. However, differences in the S_1 state still remain to be revealed.