THE RAMAN EXCITATION PROFILE SPECTRUM OF B-CAROTENE

Dissertation for the Degree of Ph. D. MICHIGAN STATE UNIVERSITY ROBERT JAMES THRASH 1977



This is to certify that the

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ABSTRACT

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By

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The Raman excitation profile spectrum of β -carotene was obtained in the energy range 16,900 cm⁻¹ to 19,000 cm⁻¹. This covers the region from the low energy tail of the strong visible absorption to where preresonance Raman effects become small. The spectrum, measured at room temperature in cyclohexane, consists of five features appearing at $18,830 \text{ cm}^{-1}$, $18,710 \text{ cm}^{-1}$, $18,600 \text{ cm}^{-1}$, $18,380 \text{ cm}^{-1}$, and 18,280 cm⁻¹. The two stronger features were assigned as carbon-carbon double bond stretching (18,830 cm^{-1}) and carbon-carbon single bond stretching (18,380 cm⁻¹) modes, while the remaining features were assigned as carbon-hydrogen It is suggested that these five features bending modes. all belong to a low-lying ${}^{l}A_{\alpha}$ electronic state in β -carotene (C_{2h} symmetry assumed). The origin of this state could not be found experimentally, but it is expected to be at 17,230 $\pm 100 \text{ cm}^{-1}$.

This low-lying ${}^{1}A_{g}$ state may play an important role in photosynthesis. β -carotene is known to serve as an accessory pigment, transferring excitation energy to chlorophyll. If the energy transfer occurs via a Förster-type mechanism, then this low-lying state must be involved.

THE RAMAN EXCITATION PROFILE SPECTRUM OF β -CAROTENE

Ву

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This work is dedicated to my wife, Pat, without whose support it would not have been possible.

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TABLE OF CONTENTS

																					Page
LIST OF	TABLES	5.	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	v
LIST OF	FIGUR	ES.	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	vi
CHAPTER	I.	Int	tro	odu	ct	10	n	•	•	•	•	•	•	•	•	•	•	•	•	•	1
CHAPTER	II.	The	eoi	ry	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	6
CHAPTER	III.	Exp	pei	rim	en	ta	.1	•	•	•	•	•	•	•	•	•	•	•	•	•	14
		cv	n I	Dye	L	as	er	•	•	•	•	•	•	•	•	•	•	•	•	•	14
		cv	N I	Dye	L	as	er	A	11	gr	nme	nt	•	•	•	•	•	•	•	•	18
		Mo	ond	och	ro	ma	to	r	an	d	As	sc	ci	lat	ed	l F	'ar	rts	5.	•	22
		E	k pe	eri	me	nt	al	F	'ro	oce	edu	re	•	•	•	•	•	•	•	•	30
CHAPTER	IV.	Res	su:	lts	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	42
CHAPTER	v.	Dis	scı	uss	io	n	•	•	•	•	•	•	•	•	•	•	•	•	•	•	54
]	Int	ter	pr	et	at	ic	n	•	•	•	•	•	•	•	•	•	•	•	54
		(Cor	npa	ri	so	n	W1	.th	ı C)th	er	·F	°0]	lye	ene	s	•	•	•	76
]	Emp	p li	ca	ti	on	S	of	' t	;hi	S	lĄ	٩	St	at	e	•	•	•	78
		S	Sug	gge	st	10	ns	f	`or	F	ut	ur	e	ь Wc	ork	ζ.	•	•	•	•	81
APPENDI	ΚΑ.		•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	84
APPENDI	(в.		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	87
APPENDIX	кс.		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	88
REFERENC	CES .		•	•	•	•		•	•	•	•	•		•	•	•	•	•	•	•	98

LIST OF TABLES

Table	Pa	age
I.	Sample Excitation Profile Parameters	
	and Calculated Points	12
II.	Tuning Ranges for the Dyes Useful in	
	the cw Dye Laser	17
III.	Parameters and Calculated Points of	
	the Six State Excitation Profile	
	Calculation	59
IV.	Parameters and Calculated Points of	
	the Seven State Excitation Profile	
	Calculation	62
v.	Parameters and Calculated Points of	
	the Seven State Calculation Using	
	$\alpha_6 = \alpha_7 = 10^5 \dots \dots$	66
VI.	Energies of the Observed Excitation	
	Profile Features and Their Differences	71
VII.	Comparison With Other Polyenes	77

LIST OF FIGURES

Figure	Pag	ςe
1	β -Carotene	2
2	Sample Excitation Profile Calculation	13
3	Tunable Dye Laser Optical Cavity	
	Configurations	6
4	Power Output versus Wavelength Using	
	Sodium Fluorescein with the 50%	
	Reflectivity Output Mirror	19
5	Correct Image of M2 on Card (2x)	21
6	Sample Housing	24
7	Block Diagram of the Experimental	
	Apparatus	25
8	Wavelength Voltage Bucking Control	
	Circuit	27
9	бО Hz Notch Filter	28
10	Current Amplifier and Voltage	
	Integrator	29
11	Beer's Law Plots at 512 nm and	
	522 nm	32
12	Typical Point Using Electronic	
	Integration	35
13	Typical Point Using the Digital	
	Computer	38
14	Typical Point Using Triangulation	10

Figure

15	Excitation Profile Obtained by
	Electronic Integration, with a
	Portion of the Visible Absorption
	Spectrum
16	Visible Absorption Spectra of
	β -carotene at High and Low
	Concentrations
17a	Visible Absorption Spectra of
	β -carotene Before (-) and After ()
	an Excitation Profile Experiment 47
17b	Visible Absorption Spectra of Fresh
	(-) and Two-Week Old () β -carotene
	Solutions
18	Excitation Profile Obtained Using
	the Digital Computer
19	Excitation Profile Obtained by
	Triangulation
20	Six State Calculation Using α_6 =
	2000
21	Seven State Calculation Using
	$\alpha_6 = \alpha_7 = 200061$
22	Seven State Calculation Using
	$\alpha_6 = \alpha_7 = 10^5 \dots \dots$
23	Excitation Profile of β -carotene
	in the 0-0 Region
24	Lycopene and Isorenieratene 82

CHAPTER I

INTRODUCTION

The class of molecules known as linear polyenes, that is molecules consisting of an unbranched chain of alternating carbon-carbon single and double bonds, possesses both allowed and forbidden excited electronic states. Forbidden electronic states are those excited states which are inaccessible from the ground state by the ordinary electric dipole absorption of one photon. The polyene chromophore in the β -carotene molecule may be considered as having a center of symmetry despite the possible absence of this symmetry element in the molecule as a whole (see Figure 1). This symmetry manifests itself in the electronic states by providing even or odd parity to the wavefunctions with respect to coordinate inversion. Group theory gives selection rules for ordinary onephoton absorption which require $g \rightarrow u$ or $u \rightarrow g$ transitions while forbidding g + g or u + u transitions.¹ Since the ground state of the polyene molecules is a g state,² then those excited electronic states which are also g states are the forbidden states referred to above. Electronic states which are not accessible from the ground state due to spin multiplicity changes will not be dealt with in detail. In Chapter V it will be shown that spin forbidden transitions cannot play a significant role in

Virtual Center of Symmetry

Figure 1. β -carotene.

the experiments described in this thesis. Since most polyene ground states are singlet states, the forbidden states to be discussed are excited singlet g states of β -carotene.

Since the 1930's there has been considerable doubt whether the lowest excited singlet state of polyene molecules has g or u symmetry.³ Early molecular orbital theory calculations, which agreed well with observed absorption spectra, predicted the lowest excited singlet state to be a u (therefore allowed) state. The emission properties of these molecules did not support this claim. Large fluorescence Stokes shifts and long fluorescent lifetimes seemed to indicate the presence of some unobserved singlet state lower in energy than the strongly allowed u state. Recent experimental work on diphenyloctatetraene and similar molecules has demonstrated the existence of such a state of sufficiently low transition amplitude to be forbidden, hence a g state.⁴⁻⁷

 β -carotene is a linear polyene of considerable

biological importance. Complete knowledge of its excited electronic state properties would be helpful in evaluating its role in photosynthesis. Because of the encouraging results obtained on the other polyene molecules and for its biological implications, β -carotene was chosen as a molecule in which to look for a low energy g state.

Group theory predicts selection rules for two-photon absorption spectroscopy which allow g - g and u - u transitions while forbidding g - u and u - g transitions.⁸ As a result, the observation of two-photon absorption processes would be an ideal way to locate this type of forbidden electronic state. For molecules in dilute solution the best method for detecting a two-photon absorption process is to monitor two-photon induced fluorescence.⁹ This technique is useful primarily because of its high sensitivity even when the sample is in low concentration. However, there is recent evidence to indicate that β -carotene does not fluoresce when excited in the visible.¹⁰ and that the fluorescence which was previously reported is likely to be from impurities. The direct observation of two-photon absorption could also be a possible technique for observing the desired forbidden electronic states. However, these experiments generally require very high sample concentrations and suffer the low sensitivities inherent in an absorption experiment. In view of the possible impurity interference and inability to observe two-photon induced fluorescence or direct absorption (due to

necessarily low sample concentrations of less than 10^{-2} M), it became necessary to use a different technique to study β -carotene.

An alternative technique for seeking low energy forbidden electronic states of the type expected for β -carotene is to examine the Raman excitation profile spectrum of the system.¹¹ An excitation profile is obtained when one plots the Raman scattering intensity as a function of the excitation energy. This results in a graph which has the appearance of an absorption spectrum, but need not reproduce the ordinary absorption spectrum and contains additional information. In particular, the excitation profile may contain features indicating the presence of forbidden electronic transitions. A more complete description of an excitation profile spectrum is given in the next chapter.

The resonance Raman spectrum of β -carotene is well known,¹² as is its excitation profile in the region of the strongly allowed visible transition.¹³ It is the purpose of this work to examine the excitation profile of β -carotene at energies somewhat lower than the strongly allowed state, in order to seek a forbidden electronic state in this energy region. This is the first work on the excitation profile spectrum of β -carotene in the preresonance region.

Chapter II of this thesis briefly describes the theory necessary to understand the appearance of forbidden

transitions in the excitation profile. Chapter III discusses the experimental methods used in obtaining the excitation profiles and Chapters IV and V present and discuss the results of this study.

CHAPTER II

THEORY

Approximate formulas have been derived to demonstrate the appearance of forbidden electronic states in the excitation profile spectrum.^{11,14} These formulas also provide a method for estimating what the molecular parameters must be in order to observe the forbidden state with an acceptable signal-to-noise ratio. An outline of the derivation and a discussion of the assumptions involved will be given here (from Friedman and Hochstrasser, Reference 14).

The amplitude for Stokes Raman scattering from a molecule originating in its ground electronic and vibrational state is proportional to $R_{0 \rightarrow V}$ where:

$$R_{0 \to V} = \sum_{j} \frac{\alpha_{j}}{\Delta \varepsilon_{j} + \frac{1}{2} \Gamma_{j}} . \qquad 1$$

The subscripts o and v indicate the vibrational quantum numbers of the initial and final states, respectively. The energy differences $\Delta \varepsilon_1$ are:

$$\Delta \varepsilon_{j} = \varepsilon_{j} - \varepsilon_{p} \qquad 2$$

where ε_j is the energy of the jth electronic state and ε_p is the energy of the exciting photon. The Γ_j are the

damping factors and the α_i are given by:

$$\alpha_{j} = \langle \mathbf{v} | \boldsymbol{\mu} | \boldsymbol{j} \rangle \langle \boldsymbol{j} | \boldsymbol{\mu} | \boldsymbol{o} \rangle.$$

The quantity α_j is the geometric mean of the dipole strengths for the transitions connecting the initial and final states with the intermediate state, j.¹⁴ For clarity and mathematical simplicity it is initially desirable to assume that only two states are important in the summation over all the excited electronic states. This is equivalent to assuming that the forbidden state, which is being sought, and a nearby allowed electronic state are the only ones sufficiently close in energy to the exciting photon energy to be important contributors to the scattering intensity. This reduces equation 1 to:

$$R_{0+v} = \frac{\alpha_1}{\Delta \varepsilon_1 + \frac{1}{2}i\Gamma_1} + \frac{\alpha_2}{\Delta \varepsilon_2 + \frac{1}{2}i\Gamma_2} . \qquad 4$$

Here the subscript 1 refers to the term involving the forbidden electronic state and the 2 refers to the term involving the nearby allowed state. The Raman scattering intensity is related to the (complex) amplitude squared, which is:

$$|\mathbf{R}_{0+\mathbf{v}}|^{2} = \left(\frac{\alpha_{1}}{\Delta \varepsilon_{1} + \frac{1}{2}\mathbf{i}\Gamma_{1}} + \frac{\alpha_{2}}{\Delta \varepsilon_{2} + \frac{1}{2}\mathbf{i}\Gamma_{2}}\right) \left(\frac{\alpha_{1}^{*}}{\Delta \varepsilon_{1}} - \frac{1}{2}\mathbf{i}\Gamma_{1}} + \frac{\alpha_{2}^{*}}{\Delta \varepsilon_{2}} - \frac{1}{2}\mathbf{i}\Gamma_{2}\right).$$

7

Expansion of Equation 5 produces four terms:

$$|\mathbf{R}_{0 \to \mathbf{V}}|^{2} = \frac{\alpha_{1}^{2}}{\Delta \varepsilon_{1}^{2} + \frac{1}{4}\Gamma_{1}^{2}} + \frac{\alpha_{2}^{2}}{\Delta \varepsilon_{2}^{2} + \frac{1}{4}\Gamma_{2}^{2}} \qquad 6a$$

+
$$\frac{\alpha_{1}\alpha_{2}^{*}}{\Delta \varepsilon_{1}\Delta \varepsilon_{2} + \frac{1}{4}\Gamma_{1}\Gamma_{2} + \Delta \varepsilon_{2}\frac{1}{2}\Gamma_{1} - \Delta \varepsilon_{1}\frac{1}{2}\Gamma_{2}} \qquad 6b$$

+
$$\frac{\alpha_1^{*}\alpha_2}{\Delta \varepsilon_1 \Delta \varepsilon_2 + \frac{1}{4}\Gamma_1\Gamma_2 + \Delta \varepsilon_1 \frac{1}{2}i\Gamma_2 - \Delta \varepsilon_2 \frac{1}{2}i\Gamma_1}.$$
 6c

The two terms in part 6a are in their final form, but terms 6b and 6c may be reduced to:

$$\frac{\alpha_{1}\alpha_{2}^{*}(\Delta\epsilon_{1}\Delta\epsilon_{2} + \frac{1}{4}\Gamma_{1}\Gamma_{2} + \Delta\epsilon_{1}\frac{1}{2}i\Gamma_{2} - \Delta\epsilon_{2}\frac{1}{2}i\Gamma_{1})}{D} \qquad 6b'$$

and

$$\frac{\alpha_{1}^{*}\alpha_{2}(\Delta\epsilon_{1}\Delta\epsilon_{2} + \frac{1}{4}\Gamma_{1}\Gamma_{2} + \Delta\epsilon_{2}\frac{1}{2}i\Gamma_{1} - \Delta\epsilon_{1}\frac{1}{2}i\Gamma_{2})}{D} \qquad 6c'$$

where D is given by:

$$D = (\Delta \varepsilon_1^2 + \frac{1}{4} \Gamma_1^2) (\Delta \varepsilon_2^2 + \frac{1}{4} \Gamma_2^2). \qquad 7$$

Adding terms 6b' and 6c' gives two new terms:

$$\frac{(\alpha_1\alpha_2^{*} + \alpha_1^{*}\alpha_2) (\Delta \varepsilon_1 \Delta \varepsilon_2 + \frac{1}{4}\Gamma_1\Gamma_2)}{D}$$
 8a

$$\frac{\alpha_{1}\alpha_{2}^{*}(\Delta\epsilon_{1}\frac{1}{2}i\Gamma_{2}-\Delta\epsilon_{2}\frac{1}{2}i\Gamma_{1}) + \alpha_{1}^{*}\alpha_{2}(\Delta\epsilon_{2}\frac{1}{2}i\Gamma_{1}-\Delta\epsilon_{1}\frac{1}{2}i\Gamma_{2})}{D} \cdot 8b$$

All of the terms needed for $|R_{0 \rightarrow V}|^2$, which are 6a, 8a, and 8b, must be real due to the absolute square. However, if it is now assumed that α_1 and α_2 are real, then the term 8b vanishes leaving

$$|R_{0+v}|^{2} = \frac{\alpha_{1}^{2}}{\Delta \varepsilon_{1}^{2} + \frac{1}{4}\Gamma_{1}^{2}} + \frac{\alpha_{2}^{2}}{\Delta \varepsilon_{2}^{2} + \frac{1}{4}\Gamma_{2}^{2}} + \frac{(\Delta \varepsilon_{1} \Delta \varepsilon_{2} + \frac{1}{4}\Gamma_{1} \Gamma_{2})(2\alpha_{1}\alpha_{2})}{(\Delta \varepsilon_{1}^{2} + \frac{1}{4}\Gamma_{1}^{2})(\Delta \varepsilon_{2}^{2} + \frac{1}{4}\Gamma_{2}^{2})} \quad 9$$

Taking the exciting photon energy to be resonant with the forbidden electronic state, the situation of importance in the current experiments, gives the three terms the following significance:

The first term is a resonance Raman term, but it will contribute very little to the scattering intensity since α_1 is very small in comparison to α_2 because of the forbidden nature of α_1 . The second term is a preresonance Raman term arising from the nearby allowed electronic state and is the major contributor to the Raman scattered intensity at this exciting photon energy. The third term is referred to as an interference term.¹⁴ This is the term which will produce a feature in the excitation profile indicating the forbidden state. It is of significant magnitude due to the cross product $\alpha_1\alpha_2$.

It is useful to obtain the ratio of the interference

and

(I) term to the preresonance (P) term. This ratio provides a means of estimating the parameters which will produce an observable feature in the excitation profile spectrum. (The resonance term containing α_1^2 is neglected since $\alpha_2 >> \alpha_1$.)

$$I/P = \frac{\frac{(\Delta \varepsilon_{1} \Delta \varepsilon_{2} + \frac{1}{4} \Gamma_{1} \Gamma_{2})(2\alpha_{1}\alpha_{2})}{(\Delta \varepsilon_{1}^{2} + \frac{1}{4} \Gamma_{1}^{2})(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2})}{\frac{\alpha_{2}^{2}}{\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2}}}$$
10

This expression reduces to:

$$I/P = \frac{2\alpha_1(\Delta\epsilon_1\Delta\epsilon_2 + \frac{1}{4}\Gamma_1\Gamma_2)}{\alpha_2(\Delta\epsilon_1^2 + \frac{1}{4}\Gamma_1^2)} \qquad 11$$

Taking γ as the signal-to-noise ratio, I/P should be greater than γ^{-1} for a significant feature to be observed. Let $\alpha_2/\alpha_1 = 10^4$, then (neglecting the Γ 's) the effect will be observed if

$$\frac{\Delta \epsilon_2}{\Delta \epsilon_1} > 10^4 / 2\gamma. \qquad 12$$

If the exciting radiation is resonant with α_1 (that is, taking $\Delta \varepsilon_1 \approx \Gamma_1$), then interference will be detected when

$$\Delta \epsilon_2 > 10^4 \Gamma_1 / 2\gamma.$$
 13

If $\gamma = 5$, then preresonance must be observed 10^3 cm^{-1} away from the allowed state (taking $\Gamma_1 = 1 \text{ cm}^{-1}$) in order to observe interference with a forbidden state at that energy. The values substituted in these equations are only approximate and used here for demonstration purposes. Later these expressions will be employed to reproduce the observed excitation profile spectrum for β -carotene.

Using Equation 9 it is possible to determine the expected appearance of a Raman excitation profile spectrum. To this end a program was written for an HP25C calculator (given in Appendix A) to evaluate Equation 9 for various values of the parameters. The result of the calculation for the parameters listed in Table I is shown in Figure 2. Since the experimentally determined excitation profile is obtained by plotting individual points (each point being a measure of the Raman scattered intensity from β -carotene at a specific exciting photon energy), Figure 2 is a good approximation to the actual excitation profile.

$\alpha_1 = 1$ $\alpha_2 = 1000$		$\Gamma_1 = 30 \text{ cm}^{-1}$ $\Gamma_2 = 100 \text{ cm}^{-1}$	l -1	ε ₁ = 18,80 ε ₂ = 20,70	0 cm ⁻¹ 0 cm ⁻¹
$\epsilon_{p}(cm^{-1})$	R ²	$\epsilon_{p}(cm^{-1})$	r ²	$\epsilon_{p}(cm^{-1})$	R ²
18600	.231	18760	.289	18920	.306
610	.234	770	.297	930	.311
620	.236	780	.307	940	.315
630	.239	790	.310	950	.319
640	.242	18800	.283	960	.323
650	.244	810	.252	970	.327
660	.247	820	.251	980	.331
670	.250	830	.258	990	.336
680	.253	840	.266	19000	.340
690	.256	850	.273		
18700	.260	860	.278		
710	.263	870	.284		
720	.267	880	.289		
730	.272	890	.293		
740	.277	18900	.298		
750	.282	910	.302		

Table I. Sample Excitation Profile Parameters and Calculated Points.



Figure 2. Sample Excitation Profile Calculation.

CHAPTER III

EXPERIMENTAL

An excitation profile spectrum is obtained by measuring the Raman scattering of the sample excited by numerous different photon energies in the region of interest. The plot of the scattering magnitude as a function of incident photon energy is the excitation profile. Clearly some arrangement must be made to obtain the Raman spectrum of the sample at many pumping energies.

cw Dye Laser

The excitation source typically used for Raman spectroscopy is a high power gas laser, such as an Argon ion laser. The several wavelengths available from these gas lasers can be used to obtain excitation profile data, but they have obvious tuning limitations. The wavelength region of interest for β -carotene is from 510 nm to 580 nm. This covers the range from the low energy tail of the absorption spectrum to the point where preresonance effects become very small. Since there are only a few gas laser lines in this range it was necessary to construct a continuous wave (cw) tunable organic dye laser for this investigation. Though this cw dye laser is similar to

units which have since become commercially available, it possesses some unique features so its construction will be detailed here.

The basic three-mirror folded optical cavity is shown in Figure 3a. This geometry was chosen rather than simpler two-mirror cavities for two reasons. First, the folding mirror M2 introduces an astigmatism which can be used to correct the astigmatism introduced by the dye volume placed at Brewster's angle.¹⁵ Second, this geometry eliminates the need for passing the high intensity pumping radiation through one of the dye laser mirrors, which would require costly special optics and optical coatings.

The high intensity pumping radiation necessary to produce lasing in the dye solution is provided by a Spectra-Physics model 164 Argon ion laser. This gas laser is capable of producing four watts of output power if operated in what is referred to as the "all-lines mode". This means adjusting the optical cavity of the gas laser so that it may lase on most of its possible wavelengths simultaneously. To take full advantage of this high power multi-line pumping radiation it was necessary to use a dye flow system which would neither sustain damage due to decomposed dye nor introduce a chromatic aberation on the pumping radiation. An unconfined (that is, no dye cell) flowing jet stream is used to transport the dye solution at a high velocity through the active pumping region. A Spectra-Physics model 376 dye circulator and nozzle



Tunable Dye Laser Optical Cavity Configurations. Figure 3.

were used for this purpose. In order to maintain a uniform dye film in the active region it is necessary to use a rather viscous solvent for the dye. For the dyes used in this laser (see Table II) the best solvent is ethylene glycol. See Appendix B for dye solution preparation.

Table II. Tuning Ranges for the Dyes Useful in the cw Dye Laser.

Dye	Tuning Range
Sodium Fluorescein	525 nm to 575 nm
Rhodamine 6 G	570 nm to 610 nm
Rhodamine B	590 nm to 640 nm

Tuning of the cw dye laser is accomplished by adding a rotatable diffraction grating to the optical cavity just outside the output mirror, as shown in Figure 3b. Mounted in the Littrow configuration, the grating reflects a selected wavelength (dependent on the grating rotation) back into the laser cavity. This decreases the cavity loss at that specific wavelength, causing the dye to lase only at the chosen wavelength. By adjusting the angle of the diffraction grating the output of the dye laser can be tuned over a broad wavelength range. The dyes useful in this laser, together with their tuning

ranges, are listed in Table II. When the tuning technique described above is employed the linewidth of the cw dye laser output is approximately 0.1 nm. The combination of the mirror and grating at the output end produces an optical cavity of very low overall loss. Consequently, in order to obtain usable light output, it is necessary to insert a beam splitter into the optical cavity. This element is located between the output mirror and diffraction grating and couples out approximately ten percent of the radiation present in this part of the cavity. One feature of this overall system, which is very useful, is that the cw dye laser output power is inherently nearly constant over much of the tuning range (except near the tuning limits) without requiring complicated power adjusting circuits. The output power as a function of wavelength is shown in Figure 4. The power available for the excitation profile experiments varied between five and forty milliwatts and remained stable for a period of 10 to 30 minutes at a given wavelength.

cw Dye Laser Alignment

Since this cw dye laser is "home-made" and easily disassembled and moved, it is necessary to understand the procedure for aligning the optical cavity.

Referring to Figure 3a, the specific optical elements of this dye laser are as follows: the concave mirrors



Figure 4. Power Output versus Wavelength Using Sodium Fluorescein with the 50% Reflectivity Output Mirror.

M2 and M3 are five cm radius of curvature mirrors with high reflectivity dielectric coatings useful in the range from about 500 nm to 650 nm. The output mirror M1 is a flat mirror with either 96% reflectivity at 632.8 nm or \sim 50% reflectivity over the range from 500 nm to 600 nm. The focusing lens is achromatic and has a focal length of 2.54 cm. When these optical elements are properly selected and adjusted the dye laser cavity is suitable for any of the dyes listed in Table II.

Adjusting the optical elements is done as follows: <u>Step 1.</u> With the Argon ion laser operating at low intensity (\sim 100 mwatts) adjust the output so that it strikes the flowing dye approximately 0.5 cm from the nozzle (all other optical elements removed). <u>Step 2.</u> Adjust the angle of the dye nozzle to Brewster's angle by tilting it until the reflected laser intensity is a minimum.

<u>Step 3.</u> Insert the focusing lens in the path of the Argon ion laser beam and bring the light to a sharp focus on the dye stream.

<u>Step 4.</u> Insert the mirrors Ml and M2 in their approximate locations. Turn up the Argon ion laser to full power. The distance of M2 from the dye will be determined experimentally. The Ml distance should be about 30 cm, but it is not critical. Adjust the reflected light from M2 so that it strikes Ml in the center. Then adjust Ml so that the light strikes a

card mounted on top of the ion laser. The light on the card will produce an image which depends on the M2 distance. (It also depends on the M1 distance, but M1 should remain fixed.) Adjust the distance of M2 until the image looks like Figure 5. (Be sure to keep the reflected light striking the center of M1.)

Figure 5. Correct Image of M2 on Card (2x)

<u>Step 5.</u> This is the most difficult step. It is now necessary to insert mirror M3. The light reflected from M3 must pass through the active region of the dye film (where the pumping light is focused), then strike M2 and be reflected to M1 and thence to the card. Once the light reaches the card, all that remains is to adjust its image to be identical to that from M2 and then superimpose the two images. In doing all this, be sure not to move M2 or M1. <u>Step 6.</u> The last step is to adjust M1 so that the reflected light now strikes M2 in about the lower middle portion of the mirror and the dye laser should lase (if rhodamine 6 G is the dye being used; if

one of the others, the grating may also need to be aligned to obtain lasing).

The diffraction grating (1200 grooves/mm, blazed at 500.0 nm) is simply aligned by placing it about 20 cm behind the output mirror and adjusting the first order reflection so that it exactly reenters the dye laser. Now slight rotation about the vertical axis of the grating should change the dye laser output radiation (tuning).

Once aligned and operating, the dye laser will need only periodic minor adjustments of M3 to keep it performing at full output power. However, when restarting the laser after turning off the dye flow it will be necessary to adjust slightly all three mirrors due to minor changes in the dye flow characteristics.

Monochromator and Associated Parts

The monochromator used for dispersing the Raman scattered radiation was a Spex 1401 double monochromator with slits adjusted so that it could be used as a single monochromator to maximize throughput. The scattered radiation was detected using an RCA C31034 photomultiplier tube cooled with solid dry ice. The sample solution being studied was contained in an ordinary 1 cm spectrophotometer cuvette having all four sides optically polished. During the experiment the sample cell was placed

in a housing mounted directly on the entrance slit of the monochromator. This housing, shown in Figure 6, contained a 2.54 cm focal length collection lens which focused the 90° scattered radiation onto the entrance slit. The housing also had provisions for mounting colored glass filters on the entrance and exit holes in order to control stray radiation. The laser light was focused into the sample by means of a 10 cm focal length lens mounted a short distance from the sample housing. A complete block diagram of the experimental apparatus is shown in Figure 7.

The data involve two electrical signals. One signal comes from the monochromator and provides a voltage which is proportional to the wavelength passing through the exit slit. The second is the current signal from the photomultiplier tube, which is proportional to the scattered light intensity. Two electronic circuits were designed to handle these signals and make them compatible with oscilloscope or X-Y recorder displays. They are described in detail here.

The purpose of the wavelength voltage bucking control circuit is as follows. The wavelength drive of the Spex 1401 monochromator controls the slide of a variable potentiometer. When the proper voltage is applied across the potentiometer, the voltage measured from the slide gives the wavelength, where one millivolt corresponds to 0.1 nm. In the wavelength range of interest, this



Front View



Figure 6. Sample Housing.



Figure 7. Block Diagram of the Experimental Apparatus.

corresponds to approximately six volts. However, during data collection only small changes in wavelength are made (approximately 10 nm) which produce only small changes in a large DC voltage. Thus the main purpose of the bucking control circuit is to remove that large DC voltage, leaving the voltage changes to be recorded. This is accomplished by adding a bucking voltage to that produced by the wavelength potentiometer sufficient to set the total voltage nearly equal to zero; thus only the voltage difference is detected on changing the wavelength. Since a wavelength change of 10 nm produces a voltage change of only 100 mV, the bucking control circuit also contains an amplifier with a gain of 10. The circuit has a provision for precisely adjusting the voltage supplied to the wavelength potentiometer as well as a control to set the bucking zero point. The schematic diagram for this circuit is shown in Figure 8. The circuit connects directly to the wavelength potentiometer of the monochromator and its output leads to the X input of an X-Y recorder through a 60 Hz notch filter. This filter was inserted in the circuit to eliminate the 60 Hz noise which is picked up along the great lengths of wire connecting the various components. The schematic diagram for this filter is shown in Figure 9.

The most important electronic circuit in this experiment is the current amplifier-voltage integrator. Shown schematically in Figure 10, it consists of two major






Figure 9. 60 Hz Notch Filter.



Figure 10. Current Amplifier and Voltage Integrator.

The current coming from the phototube is treated parts. by the first part of the circuit to produce a voltage signal which is proportional to the scattered light intensity. This voltage signal can be sent directly to the Y input of the recorder for the purpose of recording the normal Raman spectrum of the sample. However, at the option of the experimenter a switch can be thrown, sending the voltage signal to the second part of the circuit which electronically integrates the voltage signal. This integrated signal, when recorded, is directly proportional to the area under the Raman peak being investigated. The circuit is particularly important because it is this peak area which is the experimental quantity being mea-The next section describes the use of all of these sured. elements in obtaining the experimental data.

Experimental Procedure

The β -carotene used in these experiments was the synthetic, all-trans form obtained from Sigma Chemicals Company. It was stored in a dark bottle under nitrogen, in a freezer, and used without further purification. Thin-layer chromatography revealed one fluorescent impurity in the sample. The solvent for all the solutions was spectro-grade cyclohexane, also used without further purification. Solutions of β -carotene in cyclohexane of sufficient concentration for the Raman excitation

profile experiments were prepared in the following way. An excess of β -carotene was added to a small quantity of cyclohexane (approximately 25 ml.). After dissolution for about 15 minutes in darkness the colored solution was transferred by pipet to the sample cuvette, with special care being taken not to transfer any undissolved β -carotene. At this point the visible absorption spectrum was obtained as a means of determining the sample concentration. Figure 11 shows two Beer's law plots at different wavelengths (512 nm and 522 nm). The slopes (determined by least squares) were used to calculate the sample concentrations. The concentrations ranged between 7 x 10^{-4} M to 1 x 10^{-5} M in β -carotene. A fresh sample was always prepared just prior to performing an experiment. The cuvettes used in these experiments were equipped with tight fitting Teflon stoppers for two reasons. First, the cyclohexane would otherwise evaporate rapidly, thereby changing the sample concentration during the course of an experiment. Second, nitrogen gas was bubbled through each sample for five minutes just prior to each experiment in an effort to reduce the effect of dissolved oxygen, which is to enhance singlet-triplet transitions. After sample preparation in this manner the cuvette was placed in the sample housing to minimize the effects of room light.

As mentioned earlier, the Raman excitation profile spectrum consists of a plot of the Raman scattering



Figure 11. Beer's Law Plots at 512 nm and 522 nm.

intensity as a function of excitation photon energy. More specifically, what is plotted is the intensity of a selected B-carotene Raman transition as a function of excitation photon energy. In order to obtain a value of this intensity which is independent of instrumental contributions it is necessary to record the ratio of the measured g-carotene band intensity to that of some internal standard. Several instrumental effects are eliminated by this procedure. The scattered Raman intensity depends on the intensity of the source radiation. The cw dye laser pumping radiation does not have constant power as a function of the tuning wavelength. An internal standard Raman band would experience the same laser intensity dependence as the β -carotene scattering, so a ratio of the two eliminates that dependence. Other factors, such as extent of focusing, which affect the apparent pumping intensity, are also eliminated by the ratio. If the difference in wavelength of the two bands being considered is very small, then this procedure will also eliminate factors which directly influence the apparent scattered intensity. Among these factors are phototube response variations as a function of wavelength and monochromator throughput variations as a function of wavelength. If proper account is taken of all instrumental contributions to the sample scattering intensity, the excitation profile plot should provide only the molecular response. The internal standard used in all of these experiments is a Raman line

from the solvent, cyclohexane. Specifically, the β carotene band used is the one at $\Delta v = 1525 \text{ cm}^{-1}$ and the cyclohexane band at $\Delta v = 1447 \text{ cm}^{-1}$. At 600 nm this corresponds to a difference of 2.8 nm.

The intensity ratios actually used in drawing excitation profile plots were obtained in three different, independent ways. The reason for using several distinct techniques was to insure reproducibility in the absence of technique-dependent artifacts. The three techniques will be discussed in detail here.

The first and most often used method for obtaining the ratio of β -carotene Raman scattering intensity to cyclohexane reference intensity involved the direct use of the electronic voltage integrating circuit. Figure 12 shows typical experimental data for a single point on the excitation profile. This was obtained by first recording the normal Raman spectrum of the neighboring B-carotene and cyclohexane peaks with the amplifier-integrator circuit switched to the amplify mode. Then without changing anything but the monochromator scan speed and recorder vertical response, the amplifierintegrator was switched to integrate, and the areas under the peaks were recorded several times in quick succession. The monochromator was always scanned from long wavelength to short (that is, going over the β -carotene band first) at a speed of 4.0 on the scan control (approximately 5 nm per minute) for the normal spectrum and 30.0





(approximately 40 nm per minute) for the integration (the high scan rates were needed to minimize the effects of the dye laser fluctuations). The analysis of this type of data display is quite straightforward. The vertical change in the integrator signal in passing over the first Raman peak is directly proportional to the area under that peak (marked A in Figure 12). This length, A, is determined by averaging the starting positions (marked 1) and the ending positions (marked 2) of the integrator output trace, and then simply measuring the indicated distance, A, in millimeters. The process is repeated for the second peak, giving a value for the distance B. In order to have the complete information ready for plotting on the excitation profile graph, all that is necessary is to take the ratio A/B and to indicate the photon energy used to generate this particular pair of Raman signals. It should be pointed out here that a number of data points recorded in this manner displayed considerable fluctuations in the integrator output plots. These fluctuations were the reason for the averaging done in regions 1, 2, and 3 of Figure 12. The cause of the fluctuations remains unknown at this time. At first it was thought that thermal effects caused by the laser light were responsible. However, spinning the sample, which should have eliminated any thermal influence, resulted in no improvement. A second possibility was that the fluctuations were due to noise in the output of the cw dye laser. However,

improvement was also not observed when the same type of experiment was performed using the very stable output of the ion laser directly. The fluctuations did not cause any special difficulty and are not considered further.

The second and most sophisticated method employed for determining the necessary ratio involved the use of a Digital Equipment Corporation PDP 8/I mini-computer. The computer software and the accessory electronic devices used in the data acquisition were provided by a separate research group and are very well described in Reference 16; thus only the modifications and use will be detailed here. In this method the output from the current amplifier was sent to the input of a voltage-to-frequency converter. One such device was set up to produce a frequency signal proportional to the intensity of the Raman scattered radiation and a second was used to produce a frequency proportional to the dye laser intensity. These two signals were sent to a computer interface in which they were counted and these digital data were input to the computer. The computer then divided the Raman counts by the dye laser counts, thereby providing a signal as a function of time which was proportional to the Raman scattered intensity and independent of the time variations in the dye laser intensity. Since the monochromator was scanned in time, the computer time is proportional to wavelength. A typical output plot of such an experiment is shown in Figure 13. As before, one of these plots was obtained



Time (Arbitrary Units)



at every dye laser wavelength needed to produce an excitation profile spectrum. In Figure 13 each point has associated with it a number of counts. Since the points are separated by unit time periods, the area under each peak is simply the sum of the count values for the points making up the peak. This data collection technique was not often used because of an overwhelming increase in the time needed for data collection and evaluation, and because of delays caused by lack of availability of computer time. However, excitation profiles obtained with this technique were consistent with those obtained using electronic integration.

The third and simplest data collection technique consisted merely of carefully recording the Raman spectrum at each dye wavelength (several times, each over the other, to insure adequate reproducibility and proper average) and then measuring the area under each peak by triangulation. Figure 14 shows the result of a typical experimental run. This technique was chosen because it removes all electronic area measuring artifacts, but it was rarely employed because of the subjectiveness in the manual area determinations. Again, the excitation profiles obtained in this way were consistent with those obtained by the other methods.

One instrumental factor that could not be eliminated is the fact that the monochromator is linear in wavelength not wave number. At first, this seems like cause



Figure 14. Typical Point Using Triangulation.

for concern since the peak areas should be determined as a function of wave number. However, since the peaks were so narrow, the deviation from linearity (of the energy representation) is less than one percent and can be ignored. Thus having the display linear in wavelength poses no problem.

In Chapter IV of this thesis an excitation profile spectrum obtained by each of these methods will be shown. The rationale behind the choice of the particular experimental parameters (<u>e.g.</u>, slit width) will be discussed in that section.

CHAPTER IV

RESULTS

The Raman excitation profile spectrum of β -carotene reveals five basic features which are different from any feature observed in the ordinary (one-photon) visible absorption spectrum. These new features are peaks in the excitation profile spectrum at 18,830 cm⁻¹, 18,710 cm⁻¹, 18,600 cm⁻¹, 18,380 cm⁻¹, and 18,280 cm⁻¹. The remainder of this chapter will be devoted to describing in detail how each of the three methods were used to demonstrate the existence of these features, and what other experiments were done to insure that they are indeed due to β -carotene.

Figure 15 shows a plot of the excitation profile in the range from 17,600 cm⁻¹ to 19,000 cm⁻¹, obtained using the amplifier-integrator circuit. Included in the figure is a portion of the ordinary visible absorption spectrum for comparison. The features mentioned above at 18,830 cm⁻¹, 18,710 cm⁻¹, 18,380 cm⁻¹ and 18,280 cm⁻¹ are clearly visible. (The fifth peak is too weak to be seen in this spectrum.) Each point in this plot is the result of a graph similar to Figure 12. That is to say, to produce the plot of Figure 15, 59 such integrations were recorded, each having its own area ratio and corresponding exciting photon energy. (Only 57 points appear in the plot because





the points were averaged by taking a quarter of the i-1th point plus half of the ith point plus a quarter of the i + 1th point as the value for the ith point. Consequently the first and last points were dropped.)

The concentration of β -carotene used in this experiment was 5×10^{-4} M. (At concentrations below about 1×10^{-4} M the excitation profile features are lost in the background noise.) In doing experiments at such high concentrations one worries about molecular aggregation giving rise to new absorbing species, which are usually dimers, as observed in other polyene systems.¹⁷ In order to determine if such a problem is occurring in these experiments, the following test was performed. The visible absorption spectrum of g-carotene at high concentration was recorded using a 1 mm path length cuvette (in order to keep the spectrum on scale). That solution was then diluted by a factor of ten and the spectrum recorded again using a 10 mm path length cuvette. Figure 16 shows the result of this test. The fact that the two spectra appear identical indicates that no new species are formed at the higher concentration.

Another problem that may arise in these types of experiments on polyene molecules relates to the photochemical stability of the sample. Several hours of exposure to the laser radiation (even at low intensity) could produce a new absorbing species by means of cistrans isomerization. To check this possibility, the



Figure 16. Visible Absorption Spectra of β -carotene at High and Low Concentrations.

visible absorption spectrum of the sample was recorded before and after each experiment. Figure 17a shows a typical example of such a test. Again the spectra are nearly identical, indicating very little or no change in the sample during an excitation profile experiment. Figure 17b demonstrates that a sample exposed to room light for several days shows considerable change in composition. This was one of the reasons for preparing a new sample before each experiment.

Assuming that the design of the experiment eliminates influences of instrumental artifacts, and that the above tests adequately cover their respective problems, then the only remaining possible interference in the excitation profile spectrum is the presence of an impurity. The following discussion describes why impurities are not a significant problem in this type of experiment.

The resonance enhanced Raman spectrum of β -carotene has been extensively studied (see Reference 12 and references contained therein). Thus it is possible to select with confidence those bands in the Raman spectrum which are due to β -carotene. Likewise, bands due only to the solvent, cyclohexane, can be chosen with certainty. If one is very careful to measure the peak area of a band due exclusively to β -carotene and compare it to a band area due exclusively to cyclohexane, then it is extremely unlikely that an impurity could make any contribution to the excitation profile spectrum. The only way an impurity





eoredance

could still contribute would be if it had a Raman band precisely overlapping the relevant β -carotene or cyclohexane bands. Even so, this should manifest itself by altering the band shapes during the course of the experiment. Since the band shapes did not significantly change, the problem of impurities was not pursued further.

When the digital computer was used to acquire excitation profile data the spectrum shown in Figure 18 was obtained. (Only the two strongest peaks appear in this spectrum because so few points were taken.) The main motivations for using the computer to acquire data were to take advantage of its ability to integrate out temporal fluctuations in the signal, and to use the data to determine the effects of slit width on the excitation profile. The slit width effects could not be determined using the analog methods because of the extremely low light levels at narrow slit widths. In addition to collecting the data plotted in Figure 18, the computer was used to determine the g-carotene/cyclohexane peak area ratio at a constant pumping wavelength with three different slit widths: 1000 µm, 500 µm, and 200 µm. (the monochromator dispersion is 1 nm per mm). The measured area ratios at each slit width were .3799, .3927, and .4067, respectively. It is assumed that the ratio obtained with the 200 μm slit is the best value since it suffers least from peak overlap. However, 200 um slits pass too little light to get an adequate signal-to-noise ratio when analog methods are



Figure 18. Excitation Profile Obtained Using the Digital Computer.

employed. With 1000 μ m slits, the Raman signals were ample, but the peak area ratio showed considerable deviation from the 200 μ m value. Therefore, 500 μ m slits were used for all the experiments reported in this work. There was sufficient light for a reasonable signal-to-noise ratio and the deviation of the area ratio from that obtained with 200 μ m slits was small.

A third motivation for using the computer was to obtain a Raman excitation profile spectrum which was independent of any mechanical peak area measurement devices (including X-Y recorders). Presumably, the areas measured with the mini-computer are the most precise, but because of the time problem mentioned earlier the method was not often used. Since the features evident in Figure 18 are equivalent to those obtained by the other techniques, those methods were considered adequate.

Figure 19 shows the excitation profile spectrum obtained when triangulation was used to measure the peak areas. (All five features appear. These data were averaged in the same way as those of Figure 15.) For this experiment a polarization scrambler was placed just before the entrance slit of the monochromator in order to be certain that no polarization anomalies were interfering with the excitation profile. The spectrum is just like the others indicating that polarization anomalies are not interfering. The fifth and weakest feature may only appear in this spectrum because the polarization scrambler



Figure 19. Excitation Profile Obtained by Triangulation.

increases the apparent sensitivity.

The main reason for using the triangulation method was to obtain peak areas which were independent of any kind of electronic integrating device. The fact that the excitation profile spectrum shown in Figure 19 displays the same features evident in Figures 15 and 18 supports the quality of the data obtained by the other methods.

In the following chapter the discussion will center around the data shown in Figure 19, because they are the most complete. It should be remembered, however, that those data are confirmed by the results obtained by completely independent methods.

CHAPTER V

DISCUSSION

This chapter is devoted to the elaboration of the experimental results and their interpretation in terms of the excited states of 8-carotene based on the formalism of Chapter II. The chapter will be divided into four parts. The first will deal with the extensions of the material of Chapter II necessary to be useful in interpreting the β -carotene excitation profile spectrum. The second part will compare the results obtained for B-carotene with the results obtained for similar polyenes. The third section will discuss the implications of this work relating to the role of β -carotene, and carotenoids in general, in nature. Finally, the chapter will conclude with suggestions on experimental improvements and future projects. These discussions will center around the experimental conclusion that β -carotene possesses a singlet "g" state (presumably ${}^{1}A_{\sigma}$) at 17,300 ± 100 cm⁻¹.

Interpretation

The equations of Chapter II, and in particular Equation 9, which were used to calculate an expected excitation profile shape (Figure 2) can also be easily extended to reproduce the observed β -carotene spectrum in a

qualitative way. The calculation of the expected excitation profile, which involved two states (one allowed and one forbidden), produced a curve containing a single feature in the region of the forbidden state. By direct comparison, then, the minimum number of states necessary to reproduce the β -carotene excitation profile spectrum would be six, because the observed excitation profile displays five features in a region where there is no allowed transition. Anticipating the need to take additional allowed states into account, the form of equation 9 will be extended to a calculation involving seven states.

It will be convenient to rewrite Equation 9 in a very slightly different form:

$$|\mathbf{R}_{0+\mathbf{v}}|^{2} = \frac{\alpha_{1}^{2}}{\Delta\varepsilon_{1}^{2} + \frac{1}{4}\Gamma_{1}^{2}} + \frac{\alpha_{2}^{2}}{\Delta\varepsilon_{2}^{2} + \frac{1}{4}\Gamma_{2}^{2}} + \frac{(\Delta\varepsilon_{1}\Delta\varepsilon_{2} + \frac{1}{4}\Gamma_{1}\Gamma_{2})(\alpha_{1}\alpha_{2})}{(\Delta\varepsilon_{1}^{2} + \frac{1}{4}\Gamma_{1}^{2})(\Delta\varepsilon_{2}^{2} + \frac{1}{4}\Gamma_{2}^{2})} + \frac{(\Delta\varepsilon_{1}\Delta\varepsilon_{2} + \frac{1}{4}\Gamma_{1}^{2})(\Delta\varepsilon_{2}^{2} + \frac{1}{4}\Gamma_{2}^{2})}{(\Delta\varepsilon_{1}^{2} + \frac{1}{4}\Gamma_{1}^{2})(\Delta\varepsilon_{2}^{2} + \frac{1}{4}\Gamma_{2}^{2})}$$

Recalling the complex nature of the expressions involved and representing the complex form x + iy by a single letter (e.g. a) then Equation 14 can be reduced to:

$$|R_{0+v}|^2 = |a|^2 + |b|^2 + ab^* + ba^*$$
 15

which originated from

$$|R_{0+v}|^2 = (a+b)(a^{*+b^{*}}).$$
 16

Note that Equation 16 is of the same form as Equation 5. If Equation 16 represents a two state situation, then

represents the desired seven state situation. Carrying out the indicated multiplication in Equation 17 leads to:

$$|R_{0 \rightarrow v}|^2 = |a|^2 + |b|^2 + |c|^2 + |d|^2 + |e|^2 + |f|^2 + |g|^2$$

+ab#+ac#+ad#+ae#+af#+ag#+ba#+bc#+bd#

+be#+bf#+bg#+ca#+cb#+cd#+ce#+cf#+cg# +da#+db#+dc#+de#+df#+dg#+ea#+eb#+ec#+ed# +ef#+eg#+fa#+fb#+fc#+fd#+fe#+fg#+ga#+gb#

which is just like Equation 15 and thus can be used to generate all the terms necessary to write a seven-state equation just like the two-state Equation 9. It is clear that a calculation using the twenty-one pairs of crossterms and seven squared terms formed in this process would be well beyond the programming capacity of a 49 step calculator. However, a program was written for use on a HP-67 calculator (224 steps) and is given in Appendix C along with the actual form of the equation obtainable from Equation 18 used to calculate the seven state excitation profile spectra.

A calculated excitation profile spectrum taking account of five forbidden states and only one allowed state is shown in Figure 20; Table III lists the parameters and calculated points (the parameters were chosen to give a reasonable appearance). The energy chosen for the allowed state was that of the lowest energy visible absorption band, which should be near in energy to the ground vibrational level of the allowed excited electronic state. The important points to note about this calculated spectrum are first, it shows all the observed features in approximately the correct relative intensities; and second, it fails to correctly reproduce the proper background increase in slope at higher energies. This can be corrected in two ways. One way would be to increase the value of α for the allowed state. However, this is equivalent to making the forbidden states more forbidden, thereby decreasing the intensity of the five features. The second way involves the following consideration. The second peak in the visible absorption spectrum appears at 22002 $\rm cm^{-1}$, only 1300 cm⁻¹ higher in energy than the state being used in the calculation (which is ca. 1900 cm^{-1} higher than the



Figure 20. Six State Calculation Using $\alpha_6 = 2000$.

$ \alpha_1 = 2 $ $ \alpha_2 = 3 $ $ \alpha_3 = 1.5 $ $ \alpha_4 = 2 $ $ \alpha_5 = 5 $	$\alpha_6 = \alpha_7 =$ $\Gamma_1 = \Gamma_2 =$ $\Gamma_6 = \Gamma_7 =$ $\epsilon_6 = 20,$ $\epsilon_7 = 10^{49}$	2000 $\Gamma_3 = \Gamma_4 = \Gamma_5$ 200 cm ⁻¹ 700 cm ⁻¹ cm ⁻¹	= 50 c		$\begin{array}{c} 280 \text{ cm}^{-1} \\ 380 \text{ cm}^{-1} \\ 600 \text{ cm}^{-1} \\ 710 \text{ cm}^{-1} \\ 830 \text{ cm}^{-1} \\ \end{array}$
$\epsilon_{p}(cm^{-1})$	R ²	$e_p(cm^{-1})$	R ²	$\epsilon_p(cm^{-1})$	R ²
18,100	.645	18,400	.684	18,700	1.086
120	.659	420	.703	720	1.013
140	.674	440	•739	740	1.024
160	.690	460	.774	760	1.087
180	.709	480	.805	780	1.164
18,200	.730	18,500	.836	18,800	1.251
220	.756	520	.868	820	1.254
240	.787	540	.902	840	1.000
260	.816	560	.940	860	•937
280	•775	580	.976	880	.990
18,300	.725	18,600	.952	18,900	1.048
320	•755	620	.922	920	1.100
340	.804	640	.958	940	1.147
360	.845	660	1.009	960	1.190
380	.776	680	1.064		

Table III.Parameters and Calculated Points of the SixState Excitation Profile Calculation

nearest excitation profile feature). Surely if the first allowed state is involved in the excitation profile spectrum, then the second will also be important (but to a slightly lesser degree). Other, higher energy allowed states will also be involved, but to progressively smaller extents. Only the second such state will be included in these calculations because it is sufficient to approximate the observed background, and that is all that is desired. Inclusion of this second allowed state is the reason for calculating a seven-state excitation profile spectrum.

Figure 21 shows the calculated excitation profile spectrum using all seven states, and Table IV lists the parameters and calculated points. Again there are two important conclusions which may be drawn. As before, the spectrum shows all the observed features in approximately the correct relative intensities; however, now the background looks much more like the experimentally observed spectrum. Up to this point the features in the excitation profile spectrum of β -carotene have been experimentally observed and shown by means of calculation to be reasonable in appearance for forbidden transitions. What remains is to interpret these features in terms of the electronic states of β -carotene.

The five features observed in the excitation profile spectrum of β -carotene are all very sharp (half-widths of 100 cm⁻¹ or less), which indicates that they are resolved vibrational levels. For reasons to be discussed shortly,





$ \alpha_1 = 2 \alpha_2 = 3 \alpha_3 = 1.5 \alpha_4 = 2 \alpha_5 = 5 $	$\alpha_{6} = \alpha_{7}$ $\Gamma_{1} = \Gamma_{2}$ $\Gamma_{6} = \Gamma_{7}$ $\epsilon_{6} = 20,$ $\epsilon_{7} = 22,$	= 2000 = $\Gamma_3 = \Gamma_4 =$ = 200 cm ⁻¹ 700 cm ⁻¹ 002 cm ⁻¹	Γ ₅ = 50	$ \begin{array}{c} \varepsilon_1 = 1\\ \varepsilon_2 = 1\\ \varepsilon_3 = 1\\ \varepsilon_4 = 1\\ \varepsilon_5 = 1 \end{array} $	8,280 cm ⁻¹ 8,380 cm ⁻¹ 8,600 cm ⁻¹ 8,710 cm ⁻¹ 8,830 cm ⁻¹
$\epsilon_p(cm^{-1})$	R ²	$\epsilon_{p}(cm^{-1})$	R ²	$\epsilon_{p}(cm^{-1})$	R ²
18,100	1.731	18,400	1.904	18,700	2.709
120	1.761	420	1.949	720	2.604
140	1.792	440	2.019	740	2.637
160	1.826	460	2.084	760	2.751
180	1.863	480	2.146	780	2.885
18,200	1.905	18,500	2.206	18,800	3.031
220	1.954	520	2.267	820	3.034
240	2.012	540	2.332	840	2.637
260	2.064	560	2.403	860	2.564
280	2.003	580	2.469	880	2.670
18,300	1.933	18,600	2.440	18,900	2.781
320	1.992	620	2.405	920	2.880
340	2.079	640	2.475	940	2.970
360	2.150	660	2.568	960	3.055
380	2.041	680	2.666		

Table IV. Parameters and Calculated Points of the Seven State Excitation Profile Calculation.
it is likely that they are vibrational levels from a single excited electronic state. Now it must be decided to which excited electronic state they belong.

The first possibility is that the vibrational levels are a part of the strongly allowed ${}^{1}B_{u}$ state and are Franck-Condon forbidden. This can be quickly eliminated for several reasons. First, the energy difference between the highest forbidden level and the lowest allowed level (1870 cm⁻¹) does not correspond to any known vibrational separation in β -carotene. Second, and more important, is the fact that Franck-Condon forbidden levels would not be expected to show such sudden dramatic changes in intensity between neighboring bands in a progression.

If the vibrational levels do not belong to the allowed ${}^{1}B_{u}$ state (indeed, it is accepted that the ${}^{1}B_{u}$ origin is near 20,700 cm⁻¹), then two choices of electronic state remain; they are the ${}^{1}A_{g}$ and some excited triplet state. The argument against the vibrational levels belonging to a triplet electronic state is as follows: It is a good assumption that the transition giving rise to the observed vibrations originates from the β -carotene ground electronic state. The ground state is ${}^{1}A_{g}$; so if the transition is to a triplet state, it must be what is referred to as a singlet-triplet transition. For large organic molecules singlet-triplet transitions usually have oscillator strengths approximately 10^{-5} that of a normal, allowed

singlet-singlet transition.¹⁸ To decide if a singlettriplet transition is involved here, a relative measure of the allowedness of the transition is needed. (That is, relative to the nearby allowed state.) Since the α 's used in the calculations are the magnitudes of the corresponding dipole transition matrix elements, they can provide the needed information. A calculation was made using the allowed α 's approximately 10^5 times the forbidden α 's, and the result is shown in Figure 22 and tabulated in Table V. This calculated spectrum shows absolutely no structural features (not even extremely weak ones) where several should appear. This fact, coupled with considerations to be mentioned shortly, indicates that the observed vibrational levels are not part of an excited triplet state. An earlier report of a triplet state being observed in the excitation profile spectrum of naphthalene¹¹ has been challenged by Ohta and Ito.¹⁹

The final possibility, and hence the conclusion, is that the vibrational levels belong to a ${}^{1}A_{g}$ state which is lower in energy than the strongly allowed ${}^{1}B_{u}$ state. Having established this fact, the question still remains, what do each of the vibrational levels represent and where is the ground vibrational level of this ${}^{1}A_{g}$ state?

In order to properly answer this question it is necessary to discuss the mechanism that allows these vibrational levels to appear in the excitation profile spectrum. At this point it is necessary to carefully reexamine the matrix



Figure 22. Seven State Calculation Using $\alpha_6 = \alpha_7 = 10^5$.

$ \alpha_1 = 2 $ $ \alpha_2 = 3 $ $ \alpha_3 = 1.5 $ $ \alpha_4 = 2 $ $ \alpha_5 = 5 $	$a_{6} = a_{7}^{*}$ $\Gamma_{1} = \Gamma_{2}^{*}$ $\Gamma_{6} = \Gamma_{7}^{*}$ $\epsilon_{6} = 20,7$ $\epsilon_{7} = 22,0$	= 10^{5} = $\Gamma_{3} = \Gamma_{4} = 200 \text{ cm}^{-1}$ = 200 cm^{-1} = 200 cm^{-1} = 200 cm^{-1}	r ₅ = 50 c	$\varepsilon_1 = 18$ $\varepsilon_2 = 18$ $\varepsilon_3 = 18$ $\varepsilon_4 = 18$ $\varepsilon_5 = 18$,280 cm ⁻¹ ,380 cm ⁻¹ ,600 cm ⁻¹ ,710 cm ⁻¹ ,830 cm ⁻¹
$\epsilon_{p}(cm^{-1})$	R ²	$\epsilon_{p}(cm^{-1})$	R ²	ε _p (cm ⁻¹)	R ²
18,100	4107.	18,400	5061.	18,700	6439.
120	4163.	420	5138.	720	6543.
140	4219.	440	5219.	740	6656.
160	4277.	460	5301.	760	6776.
180	4337.	480	5384.	780	6900.
18,200	4398.	18,500	5469.	18,800	7028.
220	4460.	520	5557.	820	7151.
240	4524.	540	5646.	840	7259.
260	4589.	560	5738.	860	7388.
280	4650.	580	5832.	880	7529.
18,300	4713.	18,600	5924.	18,900	7674.
320	4782.	620	6017.	920	7822.
340	4855.	640	6119.	940	7974.
360	4928.	660	6224.	960	8130.
380	4994.	680	6332.		

Table V. Parameters and Calculated Points of the Seven State Calculation Using $\alpha_6 = \alpha_7 = 10^5$.

elements which play a role in the Raman excitation profile process. Rewriting Equation 3 in more detail we have

$$\alpha_{j} = \langle \Psi_{e}^{\dagger} \Psi_{v}^{\dagger} | \mu | \Psi_{e}^{\dagger} \Psi_{v}^{\dagger} \rangle \langle \Psi_{e}^{\dagger} \Psi_{v}^{\dagger} | \mu | \Psi_{e}^{\dagger} \Psi_{v}^{\dagger} \rangle$$
 19

where Ψ_{e} represents the electronic part of the wavefunction, Ψ_{v} represents the vibrational part of the wavefunction; and double prime, j, and prime refer to the initial, intermediate, and final states respectively. An examination of Equation 14 indicates that in order for a peak representing a forbidden state to appear in the excitation profile spectrum the quantity α_{j} must be different from zero for the forbidden state j. It is clear that if state j is an allowed state, then α_{j} will be a large quantity. To see how α_{j} can be non-zero (but probably very small) for a forbidden state, j, it is necessary to consider the effects of vibronic symmetry.

In general, when discussing whether a transition from the ground electronic state to some excited electronic state is allowed or forbidden one considers the integral:

$$\langle \Psi_{e}^{excited} | \mu | \Psi_{e}^{ground} \rangle$$
. 20

Here, the Ψ 's represent just the electronic wavefunctions and μ is the dipole moment operator. If this integral is different from zero, the transition is said to be allowed for electric dipole radiation. If this integral equals zero, the transition is said to be forbidden. For β -carotene, in which C_{2h} symmetry is assumed, it is easy to specify when the integral <u>must</u> be zero and when it may be different from zero, because of the inversion operation in the group. The inversion operator requires that the wavefunctions be distinguished by g (gerade) and u (ungerade) subscripts. Since the integrand must be totally symmetric in order for the integral to be non-zero, for an allowed transition one of the two wavefunctions must be g and the other u - because the dipole moment operator has u symmetry. As a first approximation Equation 20 is adequate for deciding if a transition is forbidden, but when considering very sensitive techniques or very weak transitions the full vibronic wavefunction (instead of just the electronic wavefunction) must be used, as noted in Equation 21.²⁰

Fortunately, precise knowledge of the form of the vibronic wavefunctions is not necessary for determining if the integral in Equation 21 is equal to zero, but only a knowledge of the correct symmetry of each vibronic wavefunction. The correct symmetry of a vibronic wavefunction is <u>always</u> given by the direct product of the symmetries of the electronic and vibrational wavefunctions involved (even if the product $\Psi_e \Psi_v$ is not a good representation of $\Psi_{vibronic}$).²¹ It is for this reason that the wavefunctions

used in Equation 19 were written in that particular form.

At this point several things may be stated regarding the symmetries of the vibronic functions in Equation 19. The ground state vibronic function (represented by $\Psi_{\mu}^{"}\Psi_{\mu}^{"}$) must be of g symmetry. This is because the electronic wavefunction is g (as mentioned earlier) and the vibrationless wavefunction of any state of a centrosymmetric molecule is always g. The final state vibronic function is also g, because the (ground state) electronic wavefunction is g and the vibrational wavefunction is also g. This last statement can be made because the specific vibration being observed in all these Raman excitation profile experiments is the carbon-carbon double bond symmetric stretching vibration, which is g. What remains is to determine the symmetry of the vibronic function represented by $\Psi_{e}^{j}\Psi_{v}^{j}$. Remember, however, that for a_1 to be non-zero each integral of the product in Equation 19 must be non-zero. Under the present circumstances, since $\Psi_e^{j}\Psi_v^{j}$ appears in both terms, if one is non-zero, the other will be also.

The several possibilities for the intermediate state j (represented by $\Psi_e^{j}\Psi_v^{j}$) will be discussed in turn. The first is to have the electronic part of j be the excited ${}^{1}B_u$ state of β -carotene near 20,000 cm⁻¹. Having a u electronic wavefunction makes it an allowed state by virtue of Equation 20, and this would give rise to a very large a_j . However, a small a_j is needed to account for the weak excitation profile features. If the electronic part

of j is taken to be the suspected low lying ${}^{l}A_{g}$ state, then only specific vibrational functions can be coupled with it to provide non-zero α_{j} 's. Table VI gives the energies of the observed excitation profile features and their differences, as well as an indication of their relative intensities. This information will be helpful in determining assignments for the observed vibrations.

The first, and most important, point to be made is that the vibrational wavefunctions must all be of u symmetry. This is the only way to get a vibronic symmetry of u for state j, thus giving a non-zero α_1 . This result is very interesting in that it implies a type of selection rule which directly complements the vibrational selection rules for normal one-photon or two-photon electronic spectroscopy. For normal allowed transitions from the ground state only g vibrations are observed (for centrosymmetric molecules).²² In the remainder of this discussion it is assumed that the observed vibrations are u fundamentals. However, it is possible to observe bands of u symmetry which are a combination of a g fundamental and a u fundamental. If the observed bands are really combinations (and not fundamentals), it will not significantly change the assignments or the final location of the ${}^{1}A_{g}$ origin (except to move it to slightly lower energy, perhaps 200 cm^{-1}).

The second thing to be determined for the observed vibrations is their assignment in terms of the normal

Feature	Energy (cm ⁻¹)	Intensity
1	18,280	Weak
2	18,380	Moderate
3	18,600	Very Weak
4	18,710	Weak
5	18,830	Strong
Ene	rgy Differences (cm ⁻¹)	
5-1 = 550 4	-1 = 430 3-1 = 320	2-1 = 100
5-2 = 450 4	-2 = 330 3-2 = 220	
5-3 = 230 4	-3 = 110	
5-4 = 120		

.

Table VI. Energies of the Observed Excitation Profile Features and Their Differences.

modes of the molecule. This cannot be done in a very precise way because very little information is available from the infrared and Raman spectra of B-carotene, and this information concerns the ground electronic state rather than the excited ${}^{l}A_{\sigma}$ electronic state of interest The infrared spectrum supplies information regardhere. ing the u vibrations of the molecule; but for the most part, the observed bands in the infrared spectrum are due to carbon-hydrogen vibrations.²³ It is reasonable to expect the most intense bands observed in the excitation profile spectrum to be due to carbon-carbon vibrations of the polyene chain, since they have the greatest effect on the chromophore. The similar molecules, isoprene and trans-1,3-pentadiene, show carbon-carbon double bond stretching modes in the infrared around 1600 $\rm cm^{-1}$ (it must be remembered that these correspond to vibrations in the ground electronic state).²⁴ In addition, diphenyloctatetraene shows a carbon-carbon double bond vibration around 1570 $\rm cm^{-1}$ in its Raman and fluorescence spectra (again. a ground state vibration and necessarily g).⁵ The resonance Raman spectrum of β -carotene shows several bands, including those at 1525 cm^{-1} (S), 1155 cm^{-1} (S), and 1005 cm⁻¹ (W), assigned as carbon-carbon double bond stretching, carbon-carbon single bond stretching, and carbon-hydrogen bending modes, respectively.²⁵ Again, these are ground state, g-type vibrations, but all this information can be put together to form an assignment for the observed

bands.

A careful look at the excitation profile spectrum of β -carotene (Figure 19) reveals three peaks whose relative intensities and energy separations compare well with the bands observed in the resonance Raman spectrum. These bands appear at 18,830 cm⁻¹ (S), 18,380 cm⁻¹ (M), and 18,280 cm⁻¹ (W). It is reasonable to expect the strongest band in the excitation profile spectrum to be the carbon-carbon double bond stretching mode and this is the assignment of the strong band at 18,830 cm⁻¹. The only possible check for this assignment, however, is to see if reasonable assignments can be proposed for the remainder of the bands based on this first assignment.

Following the pattern established by the resonance Raman spectrum of β -carotene, it is reasonable to assign the medium band at $18,380 \text{ cm}^{-1}$ as the carbon-carbon single bond stretching mode and the weak band at 18,280 cm⁻¹ as a carbon-hydrogen out-of-plane bending mode. It is very interesting to note that the strongest band below 2000 cm^{-1} in the infrared spectrum of g-carotene is a carbon-hydrogen bending mode at 970 cm^{-1} (approximately 600 cm^{-1} below the expected position of a carbon-carbon double bond mode).²³ It is reasonable to say that the band at 18,280 cm⁻¹ in the excitation profile spectrum is the corresponding band in the excited ${}^{l}A_{\sigma}$ state. The remaining bands in the excitation profile spectrum, at 18,600 cm^{-1} (VW) and 18,710 cm^{-1} (W), can be assigned in

a similar way. In the infrared spectrum of β -carotene there are only two other strong bands below 2000 cm⁻¹. These are at \sim 1450 cm⁻¹ and \sim 1360 cm⁻¹ (about 100 cm⁻¹ and 200 cm⁻¹, respectively, below the expected carboncarbon double bond mode). These carbon-hydrogen bending modes compare very well in relative intensity and relative separation with the excitation profile bands at 18,710 cm⁻¹ and 18,600 cm⁻¹, respectively. Again, it is reasonable to say that these excitation profile bands are the corresponding modes in the ¹A_o excited state.

These considerations complete the assignment of the observed excitation profile bands in a relative way, but it would be desirable to establish the excited ${}^{1}A_{\sigma}$ state origin in order to get absolute vibrational energies for the assigned bands. To this end, the excitation profile spectrum of β -carotene was measured in the range 16,900 cm^{-1} to 17,800 cm^{-1} (the region of interest, since 18,830 cm^{-1} minus 1,600 cm^{-1} is 17,230 cm^{-1}), and is shown in Figure 23. As can be seen, no band was observed in this energy region. The absence of a 0-0 band is not surprising, however. Recall that the vibrational wavefunction in the forbidden state must be of u symmetry. However, the wavefunction for the vibrationless state (involved in the 0-0 transition) must always be of g symmetry in a centrosymmetric molecule. Having a g vibrational part with a ${}^{1}A_{r}$ electronic part would lead to g vibronic symmetry and the a will equal zero. Unfortunate as it may be, it is reasonable



Figure 23. Excitation Profile of β -carotene in the 0-0 Region.

not to see the ${}^{1}A_{g} - {}^{1}A_{g}$ 0-0 transition. One must be content to estimate the expected location of the vibrationless level of the excited ${}^{1}A_{g}$ state (and hence the ${}^{1}A_{g} - {}^{1}B_{u}$ energy gap) from the observed excitation profile spectrum. Taking 1600 ± 100 cm⁻¹ as a reasonable energy for a carbon-carbon double bond stretching mode, then the vibrationless level of the excited ${}^{1}A_{g}$ should be 17,230 ± 100 cm⁻¹ above the ground electronic state. Using as the ${}^{1}B_{u}$ vibrationless energy level 20,700 cm⁻¹ makes the ${}^{1}A_{g} - {}^{1}B_{u}$ energy gap 3470 ± 100 cm⁻¹.

Comparison With Other Polyenes

The spectroscopic properties of polyene molecules show fairly consistent variation with an increase in the number of double bonds. It is useful to compare the observed properties of β -carotene with the available data for other polyenes in which low energy ${}^{1}A_{g}$ states have been found. Table VII lists several polyenes along with the available data. The obvious points to note in Table VII are the following: First, the ${}^{1}A_{g} - {}^{1}B_{u}$ gap steadily increases (not necessarily linearly); and second, the fluorescence quantum yield steadily decreases. Since in condensed systems fluorescence usually arises from the lowest excited singlet state, it is reasonable to associate the two trends. That is, as the energy gap increases the extent of interaction between the forbidden ${}^{1}A_{g}$ state and the allowed

Polyene	Energy Gap (cm ⁻¹)	Fluorescence Quantum Yield
diphenylbutadiene	600 ^a	.44 ^b
diphenyloctatetraene	1300 ^b ,~2000 ^c	.1 ^b
diphenyltetradecaheptaene		od
β-carotene	3470±100	0 ^e
^a See Reference 26		
^b See Reference 3		
^C See Reference 7		
đ		

Table VII. Comparison With Other Polyenes.

^dSee Reference 27

^eSee Reference 10

 ${}^{1}B_{u}$ state decreases, thus decreasing the oscillator strength of the forbidden (presumably fluorescing) state. In addition, the fluorescence quantum yield could be decreased by virtue of much greater internal conversion of energy to the now much nearer ground electronic state. The ${}^{1}A_{g}$ excited state is only six or less carbon-hydrogen stretching mode quanta above the ground state. It is possible that weak fluorescence may have occurred, even at this gap size for β -carotene, had the ground state been further away. The results presented here for the location of the lowest excited ${}^{1}A_{g}$ state in β -carotene are in line with what would be expected for a polyene of this chain length.

Implications of this ¹A_g State

The significant number of polyenes which have been shown to possess a low-energy, forbidden, singlet excited state indicates that this is a general occurrence; but that is not the most important point. This low-lying state of β -carotene (and the one presumed to exist in other carotenoids) may play a very significant role in photosynthesis.

Action spectra (in the visible region) of photosynthetic systems show high yield even at wavelengths where the primary pigments (the various chlorophylls) have little or no absorption. Indeed, in certain algae the action

spectra have been interpreted as indicating a transfer of energy from β -carotene to chlorophyll.²⁸ If it is assumed that the energy transfer from β -carotene to chlorophyll takes place via the Förster mechanism, then the low-lying ${}^{1}A_{\sigma}$ excited state must be involved.

The Förester mechanism is well described in Reference 29 so only the pertinent requirements for efficient energy transfer will be mentioned here. The efficiency of energy transfer between two molecules depends on the extent of overlap of the emission spectrum of the energy donor and the absorption spectrum of the energy acceptor, but understand that the Förster mechanism does not involve the process of fluorescence followed by absorption of radiation. The mechanism does not necessarily require both transitions (donor emission and acceptor absorption) to be dipole allowed, so absence of fluorescence from β -carotene is not a serious drawback. What must be considered is how this energy overlap is provided for the two molecules (β -carotene and chlorophyll).

Chlorophyll a shows a strong absorption band maximum at 15105 cm⁻¹ and chlorophyll b shows a strong absorption maximum at 15552 cm⁻¹ (both in ether solution).³⁰ These are dipole allowed transitions, and a coupling of an allowed transition and a forbidden one is not unreasonable in the Förster mechanism.²⁹ Fluorescence spectra of large molecules in the condensed phase generally show

strong emission (if not the maximum emission) several vibrational quanta away from the emission 0-0 band. If the vibration forming the progression is the carbon-carbon double or single bond stretch (as may be likely in β -carotene), then efficient energy transfer could still take place up to 3000 cm⁻¹ (2 or 3 quanta) lower in energy than the emission 0-0 band. If the donor state in β -carotene were the ${}^{1}B_{11}$ state (with 0-0 band at 20,700 cm⁻¹), then efficient energy transfer might take place down to 17,700 cm⁻¹. But this clearly is not in good overlap with the absorption maxima of the chlorophyll molecules, making the $^{1}B_{n}$ state an unlikely candidate for the energy transferring state. However, if the donor state in g-carotene is the low-lying ${}^{1}A_{\sigma}$ excited state (with 0-0 band near 17,230±100 cm⁻¹), then efficient energy transfer could take place to 14,200 cm⁻¹, completely covering the range of the absorption in the chlorophyll molecules. It seems likely that the lowlying ${}^{1}A_{g}$ excited state in β -carotene (and possibly other carotenoids as well) may play a very significant role in photosynthesis in terms of being active in the energy transfer process necessary to make β -carotene an accessory pigment in light harvesting. B-carotene performs other functions in living systems and these are well discussed by J. Burnett in Reference 31.

Suggestions for Future Work

The Raman excitation profile spectroscopy technique is well suited to those molecules which do not fluoresce (hence, very difficult to study by two-photon techniques) and which show considerable preresonance Raman enhancement, but only to those molecules, unfortunately. However, several molecules fit these requirements, and in particular a few carotenoids which should be studied. These are lycopene and isorenieratene, with the structures shown in Figure 24. The latter is important because it is a natural product which is a simple extension of the model compound diphenyloctatetraene. The former is important because of its similarity to β -carotene.

The instrumentation of Raman excitation profile spectroscopy is another area where considerable effort could be directed. The primary goal would be to devise a method to obtain an excitation profile spectrum on a continuous (or nearly continuous) basis (as opposed to the current point by point method), much the same way an ordinary absorption spectrum is obtained. This would be useful for two reasons. First, it should greatly reduce the time required to obtain a spectrum (typically six hours for a reasonable point density, not counting data calculating and plotting time, generally another four to six hours). Second, and more important, it would allow for direct optimization of the experimental parameters (as opposed



Lycopene



Isorenieratene

Figure 24. Lycopene and Isorenieratene.

to setting up the experiment and hoping all is well). The direct optimization would provide the best opportunity for obtaining the greatest signal-to-noise ratio.

One suggestion is to use two monochromators which are linear in wavenumber along with a tunable dye laser which can be scanned linearly in wavenumber. Collect Raman scattered radiation from the sample into both monochromators. Have one monochromator set to the solute frequency and the other to the solvent frequency, and under computer control, scan the monochromators in accord with the dye laser. The computer (also collecting the data) could then evaluate the ratio and plot the excitation profile. APPENDICES

APPENDIX A

Appendix A gives a listing of the HP-25C pocket calculator program written to perform two-state excitation profile calculations. The program calculates $|R|^2$ using Equation 9 when the parameters α_2 , Γ_1 , Γ_2 , ε_1 , ε_2 , and ε_p are stored in the proper registers (α_1 is assumed to be 1). The program automatically increments ε_p by a specified amount ($\Delta \varepsilon_p$) for each run through the calculation.

Storage Registers

 $R_0 = \alpha_2$ $R_1 = \Gamma_1$ $R_2 = \Gamma_2$ $R_3 = \epsilon_1$ $R_4 = \epsilon_2$ $R_5 = \epsilon_p$ $R_6 = \Delta \epsilon_p$ R_7 used

Program Steps

Step Number	Key Code	Instructions
01	24 06	Rcl R ₆
02	23 51 05	Sto+R ₅
03	24 03	Rcl R3
04	24 05	Rcl R5
05	41	-
06	24 04	Rcl R ₄
07	24 05	Rcl R5
08	41	_ .
09	23 07	Sto R ₇
10	61	x
11	24 01	Rcl R ₁
12	24 02	Rcl R2
13	61	x
14 14	04	4
15	71	÷

Step Number	Key Code	e Instructions
16	5	1 +
17	02	2 2
18	24 00	D Rcl R _O
19	6:	l x
20	6:	L x
21	24 0'	7 Rcl R ₇
22	15 02	\mathbf{x}^2
23	24 03	2 Rcl R ₂
24	15 0	\mathbf{x}^2
25	0	4 4
26	7	l ÷
27	5	1 +
28	23 0'	7 Sto R ₇
29	7:	1 ÷ '
30	24 0	3 Rcl R ₃
31	24 0	5 Rcl R ₅
32	4:	l –
33	15 03	\mathbf{x}^2
34	24 01	l R _c l R ₁
35	15 02	x^2
36	01	4 4
37	7:	l ÷
38	5	1 +
39	7:	l ÷
40	14 7:	3 Last x
41	15 23	2 1/x
42	5	1 +
43	24 00	D Rcl R ₀
44	15 03	x^2
45	24 0'	7 Rcl R ₇
46	73	1 ÷ '
47	5	1 +
48	13 00	GO TO 00
49	13 00	GO TO 00

All the user must do is load the program and appropriate parameters and then press R/S for each desired point (ϵ_p is automatically increased).

APPENDIX B

The dye solutions are all prepared in essentially the same way. First, a high concentration stock solution is prepared for each dye. For rhodamine 6 G and rhodamine B this simply consists of a 1×10^{-2} M solution in dry, reagent grade methanol. The sodium fluorescein stock solution is prepared by making a 1×10^{-2} M solution of fluorescein in methanol and then adjusting the pH to approximately 9 with aqueous sodium hydroxide. These stock solutions can be stored for a very long time (greater than 2 years).

The actual flowing dye solutions are prepared by experimentally adjusting the concentration. Sufficient ethylene glycol is added to the circulator to one third fill it with the solvent flowing. Now it is simply a matter of adding sufficient dye stock solution to the ethylene glycol to make the laser lase (approximated 20 ml.). Once lasing, the output power is monitored and dye stock solution is slowly added until the optimum concentration is reached. In the case of sodium fluorescein, 20 drops of cyclooctatetraene must be added along with the first portion of dye and after each 2-4 hours of operation.

APPENDIX C

Appendix C gives a listing of the HP-67 pocket calculator program written to perform excitation profile calculations for up to seven states. The program calculates $|R|^2$ using the equation generated from Equation 18, which is:

$$\begin{split} |\mathbf{R}|^{2} &= \frac{\alpha_{1}^{2}}{\Delta \varepsilon_{1}^{2} + \frac{1}{4} \Gamma_{1}^{2}} + \frac{\alpha_{2}^{2}}{\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2}} + \frac{\alpha_{3}^{2}}{\Delta \varepsilon_{3}^{2} + \frac{1}{4} \Gamma_{3}^{2}} + \frac{\alpha_{4}^{2}}{\Delta \varepsilon_{4}^{2} + \frac{1}{4} \Gamma_{1}^{2}} + \frac{\alpha_{5}^{2}}{\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2}} \\ &+ \frac{\alpha_{6}^{2}}{\Delta \varepsilon_{6}^{2} + \frac{1}{4} \Gamma_{1}^{2}} + \frac{\alpha_{7}^{2}}{\Delta \varepsilon_{7}^{2} + \frac{1}{4} \Gamma_{7}^{2}} + \frac{(\Delta \varepsilon_{1} \Delta \varepsilon_{2} + \frac{1}{4} \Gamma_{1} \Gamma_{2})(2\alpha_{1}\alpha_{2})}{(\Delta \varepsilon_{1}^{2} + \frac{1}{4} \Gamma_{1}^{2})(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2})} \\ &+ \frac{(\Delta \varepsilon_{1} \Delta \varepsilon_{3} + \frac{1}{4} \Gamma_{1} \Gamma_{3})(2\alpha_{1}\alpha_{3})}{(\Delta \varepsilon_{1}^{2} + \frac{1}{4} \Gamma_{1}^{2})(\Delta \varepsilon_{3}^{2} + \frac{1}{4} \Gamma_{3}^{2})} + \frac{(\Delta \varepsilon_{1} \Delta \varepsilon_{4} + \frac{1}{4} \Gamma_{1} \Gamma_{4})(2\alpha_{1}\alpha_{4})}{(\Delta \varepsilon_{1}^{2} + \frac{1}{4} \Gamma_{1}^{2})(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2})} \\ &+ \frac{(\Delta \varepsilon_{1} \Delta \varepsilon_{5} + \frac{1}{4} \Gamma_{1} \Gamma_{5})(2\alpha_{1}\alpha_{5})}{(\Delta \varepsilon_{1}^{2} + \frac{1}{4} \Gamma_{1}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{1} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{1} \Gamma_{6})(2\alpha_{1}\alpha_{6})}{(\Delta \varepsilon_{1}^{2} + \frac{1}{4} \Gamma_{1}^{2})(\Delta \varepsilon_{6}^{2} + \frac{1}{4} \Gamma_{6}^{2})} \\ &+ \frac{(\Delta \varepsilon_{1} \Delta \varepsilon_{7} + \frac{1}{4} \Gamma_{1} \Gamma_{7})(2\alpha_{1}\alpha_{7})}{(\Delta \varepsilon_{1}^{2} + \frac{1}{4} \Gamma_{1}^{2})(\Delta \varepsilon_{7}^{2} + \frac{1}{4} \Gamma_{7}^{2})} + \frac{(\Delta \varepsilon_{2} \Delta \varepsilon_{3} + \frac{1}{4} \Gamma_{2} \Gamma_{3})(2\alpha_{2}\alpha_{3})}{(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2})(\Delta \varepsilon_{3}^{2} + \frac{1}{4} \Gamma_{3}^{2})} \end{split}$$

$$+ \frac{(\Delta \varepsilon_{2} \Delta \varepsilon_{4} + \frac{1}{4} \Gamma_{2} \Gamma_{4})(2\alpha_{2}\alpha_{4})}{(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2})(\Delta \varepsilon_{4}^{2} + \frac{1}{4} \Gamma_{4}^{2})} + \frac{(\Delta \varepsilon_{2} \Delta \varepsilon_{5} + \frac{1}{4} \Gamma_{2} \Gamma_{5})(2\alpha_{2}\alpha_{5})}{(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{2} \Delta \varepsilon_{5} + \frac{1}{4} \Gamma_{2} \Gamma_{7})(2\alpha_{2}\alpha_{7})}{(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{2} \Delta \varepsilon_{7} + \frac{1}{4} \Gamma_{2} \Gamma_{7})(2\alpha_{2}\alpha_{7})}{(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{2}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{3} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{3} \Gamma_{7})(2\alpha_{2}\alpha_{7})}{(\Delta \varepsilon_{2}^{2} + \frac{1}{4} \Gamma_{3}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{7}^{2})} + \frac{(\Delta \varepsilon_{3} \Delta \varepsilon_{5} + \frac{1}{4} \Gamma_{3} \Gamma_{7})(2\alpha_{3}\alpha_{5})}{(\Delta \varepsilon_{3}^{2} + \frac{1}{4} \Gamma_{3}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{3} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{3} \Gamma_{7})(2\alpha_{3}\alpha_{7})}{(\Delta \varepsilon_{3}^{2} + \frac{1}{4} \Gamma_{3}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{4} \Gamma_{6})(2\alpha_{4}\alpha_{6})}{(\Delta \varepsilon_{3}^{2} + \frac{1}{4} \Gamma_{3}^{2})(\Delta \varepsilon_{7}^{2} + \frac{1}{4} \Gamma_{7}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{4} \Gamma_{6})(2\alpha_{4}\alpha_{6})}{(\Delta \varepsilon_{4}^{2} + \frac{1}{4} \Gamma_{3}^{2})(\Delta \varepsilon_{7}^{2} + \frac{1}{4} \Gamma_{7}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{4} \Gamma_{6})(2\alpha_{4}\alpha_{6})}{(\Delta \varepsilon_{4}^{2} + \frac{1}{4} \Gamma_{4}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{4} \Gamma_{6})(2\alpha_{4}\alpha_{6})}{(\Delta \varepsilon_{4}^{2} + \frac{1}{4} \Gamma_{4}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{4} \Gamma_{6})(2\alpha_{4}\alpha_{6})}{(\Delta \varepsilon_{4}^{2} + \frac{1}{4} \Gamma_{4}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{4} \Gamma_{6})(2\alpha_{4}\alpha_{6})}{(\Delta \varepsilon_{4}^{2} + \frac{1}{4} \Gamma_{4}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{4} \Gamma_{6})(2\alpha_{4}\alpha_{6})}{(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{5} \Gamma_{6})(2\alpha_{5}\alpha_{6})}{(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{6}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{5}^{2})(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})}{(\Delta \varepsilon_{5}^{2} + \frac{1}{4} \Gamma_{5}^{2})} + \frac{(\Delta \varepsilon_{4} \Delta \varepsilon_{6} + \frac{1}{4} \Gamma_{5}^{2})(\Delta$$

+
$$\frac{(\Delta \epsilon_5 \Delta \epsilon_7 + \frac{1}{4} \Gamma_5 \Gamma_7)(2 \alpha_5 \alpha_7)}{(\Delta \epsilon_5^2 + \frac{1}{4} \Gamma_5^2)(\Delta \epsilon_7^2 + \frac{1}{4} \Gamma_7^2)} + \frac{(\Delta \epsilon_6 \Delta \epsilon_7 + \frac{1}{4} \Gamma_6 \Gamma_7)(2 \alpha_6 \alpha_7)}{(\Delta \epsilon_6^2 + \frac{1}{4} \Gamma_6^2)(\Delta \epsilon_7^2 + \frac{1}{4} \Gamma_7^2)}$$

In the program it is assumed that Γ_1 through Γ_5 are equal and that $\Gamma_6 = \Gamma_7$ and $\alpha_6 = \alpha_7$. The remaining parameters are stored separately. If less than seven states are to be used either set the appropriate α 's equal to zero or set the appropriate ϵ 's equal 10⁴⁹. This program also automatically increments ϵ_p by the amount $\Delta \epsilon_p$ during each pass through the program.

Storage Registers:

 $0 = \alpha_1 \quad 1 = \alpha_2 \quad 2 = \alpha_3 \quad 3 = \text{used} \quad 4 = \text{used} \quad 5 = \text{used}$ $6 = \text{used} \quad 7 = \text{used} \quad 8 = \text{used} \quad 9 = \text{used}$

 $S_{0} = \alpha_{6} = \alpha_{7} \quad S_{1} = \frac{\Gamma_{1}}{2} + \frac{\Gamma_{5}}{2} \quad S_{2} = \frac{\Gamma_{6}}{2} = \frac{\Gamma_{7}}{2} \quad S_{3} = \varepsilon_{1}$ $S_{4} = \varepsilon_{2} \quad S_{5} = \varepsilon_{3} \quad S_{6} = \varepsilon_{4} \quad S_{7} = \varepsilon_{5} \quad S_{8} = \varepsilon_{6} \quad S_{9} = \varepsilon_{7}$ $A = \alpha_{4} \quad B = \alpha_{5} \quad C = \Delta \varepsilon_{p} \quad D = \qquad E = \varepsilon_{p} \quad I =$

Program Steps

Step Number	Key Code	Instruction
001	32 25 11	g lbl f a
002	00	0
003	33 09	Sto 9
004	35 61 00	h CF O
005	31 22 01	f GSB 1
006	35 61 00	h CF O
007	31 22 09	f GSB 9
008	31 22 14	f GSB D
009	35 61 00	h CF O
010	31 22 04	f GSB 4
011	31 22 13	f GSB C
012	35 61 00	h CF O
013	31 22 05	f GSB 5
014	31 22 12	f GSB B
015	35 61 00	h CF O
016	31 22 06	f GSB 6
017	31 22 11	f GSB A

Step Number	Key Code	Instruction
018	35 61 00	h CF O
019	31 22 07	f GSB 7
020	34 04	Rcl 4
021	32 54	g X ²
022	34 06	Rcl 6
023	32 54	g X ²
024	61	+
025	34 08	Rcl 8
026	31 22 08	f GSB 8
027	34 09	Rcl·9
028	84	R/S
029	34 15	Rcl E
030	34 13	Rcl C
031	61	+
032	33 15	Sto E
033	22 31 11	GTO f a
034	31 25 01	f 1b1 1
035	31 42	f P‡S
036	34 03	Rcl 3
037	34 15	Rcl E
038	51	-
039	34 04	Rcl 4
040	34 15	Rcl E
041	51	-
042	31 22 02	f GSB 2
043	34 00	Rcl 0
044	33 07	Sto 7
045	34 01	Rcl l
046	33 08	Sto 8
047	31 22 00	f GSB 0
048	31 42	f P‡S
049	34 05	Rc1 5
050	34 15	Rcl E
051	51	-

Step Number	Key Code	Instruction
052	31 42	f P≠S
053	33 04	Sto 4
054	34 02	Rcl 2
055	33 08	Sto 8
056	31 22 00	f GSB 0
057	31 25 14	f 1b1 D
058	31 42	f P‡S
059	34 06	Rcl 6
060	34 15	Rcl E
061	51	-
062	31 42	f P‡S
063	33 04	Sto 4
064	34 11	Rcl A
065	33 08	Sto 8
066	31 22 00	f GSB 0
067	31 25 13	f 1b1 C
068	31 42	f P‡S
069	34 07	Rcl 7
070	34 15	Rcl E
071	51	-
072	31 42	f P‡S
073	33 04	Sto 4
074	34 12	Rcl B
075	33 08	Sto 8
076	31 22 00	f GSB 0
077	31 25 12	f 1b1 B
078	31 42	f P‡S
079	34 08	Rcl 8
080	34 15	Rcl E
081	51	-
082	34 02	Rcl 2
083	34 00	Rcl 0
084	31 42	f P‡S
085	33 08	Sto 8

.

Step Number	Key Code	Instruction
086	35 53	h R‡
087	33 06	Sto 6
088	35 53	h R+
089	33 04	Sto 4
090	31 22 00	f GSB 0
091	31 25 11	f 1b1 A
092	31 42	f P ‡S
093	34 09	Rcl 9
094	34 15	Rcl E
095	51	-
096	31 42	f P ≵ S
097	33 04	Sto 4
098	31 22 00	f GSB 0
099	35 22	h RTN
100	31 25 09	f 1b1 9
101	31 42	f PZS
102	34 04	Rcl 4
103	34 15	Rcl E
104	51	-
105	34 05	Rcl 5
106	34 15	Rcl E
107	51	-
108	31 22 02	f GSB 2
109	34 01	Rcl l
110	33 07	Sto 7
111	34 02	Rcl 2
112	33 08	Sto 8
113	31 22 00	f GSB 0
114	35 22	h RTN
115	31 25 04	f 161 4
116	31 42	f P‡S
117	34 05	Rc1 5
118	34 15	Rcl E
119	51	-

Step Number	Key Code	Instruction
120	34 06	Rcl 6
121	34 15	Rcl E
122	51	· _
123	31 22 02	f GSB 2
124	34 02	Rcl 2
125	33 07	Sto 7
126	34 11	Rcl A
127	33 08	Sto 8
128	31 22 00	f GSB 0
129	35 22	h RTN
130	31 25 05	f 1b1 5
131	31 42	f P‡S
132	34 06	Rcl 6
133	34 15	Rcl E
134	51	-
135	34 07	Rcl 7
136	34 15	Rcl E
137	51	-
138	31 22 02	f GSB 2
139	34 11	Rcl A
140	33 07	Sto 7
141	34 12	Rc1 B
142	33 08	Sto 8
143	31 22 00	f GSB 0
144	35 22	h RTN
145	31 25 06	f 1b1 6
146	31 42	f P‡S
147	34 07	Rcl 7
148	34 15	Rcl E
149	51	-
150	34 08	Rcl 8
151	34 15	Rcl E
152	51	-
153	31 42	f P ≠S

.

Step Number	Key Code	Instruction
154	31 22 03	f GSB 3
155	34 12	Rcl B
156	33 07	Sto 7
157	31 22 00	f GSB 0
158	35 22	h RTN
159	31 25 07	f 1b1 7
160	31 42	f P∔S
161	34 08	Rcl 8
162	34 15	Rcl E
163	51	-
164	34 00	Rcl 0
165	34 02	Rcl 2
166	31 42	f P‡S
167	33 05	Sto 5
168	35 53	h R↓
169	33 07	Sto 7
170	35 53	h R↓
171	33 03	Sto 3
172	31 22 00	f GSB O
173	35 22	h RTN
174	31 25 02	f 1b1 2
175	34 01	Rcl l
176	31 42	f P‡S
177	33 05	Sto 5
178	33 06	Sto 6
179	35 53	h R+
180	31 25 03	f 1b1 3
181	33 04	Sto 4
182	35 53	h R+
183	33 03	Sto 3
184	35 22	h RTN
185	31 25 00	f 1b1 0
186	34 03	Rcl 3
187	34 04	Rcl 4

Step Number	Key Code	Instruction
188	71	X
189	34 05	Rc1 5
190	34 06	Rcl 6
191	71	X
192	61	+
193	34 07	Rcl 6
194	34 08	Rcl 8
195	02	2
196	71	X
197	71	X
198	71	X
199	34 04	Rcl 4
200	32 54	g X ²
201	34 06	Rcl 6
202	32 54	g X ²
203	61	+
204	81	÷
205	34 03	Rcl 3
206	32 54	g X ²
207	34 05	Rcl 5
208	32 54	g X ²
209	61	+
210	81	*
211	33 61 09	Sto + 9
212	35 71 00	h F?0
213	35 22	h RTN
214	35 82	h Last X
215	34 07	Rcl 7
216	31 25 08	f 1b1 8
217	32 54	g X ²
218	35 52	h X‡Y
219	81	*
220	33 61 09	Sto + 9
221	35 51 00	h SF O

Step Number 222	Key Code 35 22	Instruction h RTN
224	84	R/S

After the program and all the parameters are loaded the user simply presses f a for the first point and R/S for each additional point. The execution time is approximately 57 seconds for each point.
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