A STUDY OF SOME NATURALLY OCCURRING AND SYNTHETIC CIS-TRANS POLYENES

Thesis by

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In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1949

The author wishes to express his deep appreciation to Professor Zechmeister for his interest and guidance in the problems connected with this research.

The application of modern laboratory methods in stereochemistry is still in a relatively early stage of development. Thus, although the structural study of natural and synthetic polyenes has expanded in recent years, the stereochemical problems presented by these highly unsaturated compounds are by no means completely solved. Indeed, for a long time there existed some doubt as to whether the presence of such a long, conjugated double bond system would not prevent cis-trans isomerism.

The experimental evidence gathered in the study of carotenoids and synthetic polyenes in the last two decades leaves little doubt about the existence of cis-trans isomers in such systems. Their study has further emphasized the idea that the morphology of molecules plays a great part in determining their properties, and that the effect of stereochemical variations may even exceed that of structural alterations.

The present study includes experiments on some physical and biological properties of stereoisomeric polyenes and also on the isolation of a new colorless polyene whose stereochemical investigation has not yet been carried out. As will be shown, a combination of chromatographic and spectroscopic methods constitutes a very useful tool in this field.

The isolation of some new stereoisomers of lycopene is presented, and the properties of these poly-*cis*-lycopenes are correlated with their steric configuration and in particular their spectral characteristics are discussed in the light of modern theories of the nature of light absorption by carotenoids. On the basis of these theories some predictions are made as to the number of *cis* double bonds and also about the expected properties of poly-*cis* forms in general.

A comparative study of the three stereoisomeric 1,4-diphenyl-butadienes was carried out, employing methods of chromatography and spectroscopy, and some points of similarity between the spectral characteristics of these synthetic polyenes and the naturally occurring carotenoids are pointed out.

The provitamin A activity of some *cis* isomers of γ-carotene was tested in feeding experiments using chicks as well as rats and it is shown that a *cis* isomer may have a provitamin A activity as great as its all-*trans* form.

A new, colorless polyene, termed phytofluenol, was isolated and some qualitative observations of its chemical and spectral characteristics are described.

Table of Contents

-Theoretical Section-

Table of Contents

-Experimental Part-

I. ISOLATION AND STUDY OF SOME NATURALLY OCCURRING cis-CAROTENOID PIGMENTS

A. INTRODUCTION

1. Steric Possibilities of the Carotenoids

Without going into great detail, some of the reasons for believing that some forms of the carotenoids represent true cis-trans isomers should be given on a historical basis. The first example of a stereoisomeric form of a carotenoid was discovered in 1923 by Herzig and Faltis (21), who were unable to account for the chance appearance of an unexpected form of bixin ($C_{25}H_{30}O_4$). Karrer and co-workers (23) made an analogous observation in 1929, and the opinion was advanced that this was a case of cis-trans isomerism since the conversion of the naturally occurring ("labile") bixin into the stable ("new") form took place upon the addition of catalytic amounts of iodine.

Gillam and El Ridi observed the first case of isomerism in C_{40}-carotenoids in 1935 (15,16,17), and both double bond migration and geometrical isomerism were discussed by them as possibilities. Observations in other carotenoid sets were soon made (51,77,78) and in 1939 Zechmeister and Tuzson (79) introduced the use of iodine in the field of C_{40}-carotenoids to catalyze steric changes. As work progressed, Zechmeister and co-workers (52,53,60,79) accumulated increased evidence for the cis-trans character of a number of phenomena.

The initial, tentative assumption of double bond migrations had to be abandoned on the following basis. a) The observed reversible changes are spontaneous in many instances or are effected under very mild conditions. b) Iodine, which, since Anschütz's work (1), became a well-known catalyst for cis-trans rearrangements, is effective in the field of

the carotenoids. c) Some carotenoid pigments, whose chromophore is blocked on both ends by conjugated keto groups (53), show the same phenomenon of a reversible isomerism, although double bond migration could not take place here unless this migration could be into some of the methyl side chains. d) The large number of isomers observed in some cases, e.g. β-carotene (65), cannot be accounted for by any double bond migration processes. e) If double bond migration occurred, an easy conversion of α-carotene ($C_{40}H_{56}$) to β-carotene ($C_{40}H_{56}$) and of lutein ($C_{40}H_{56}O_2$) to zeaxanthin ($C_{40}H_{56}O_2$) would be expected to take place. Such conversions have never been observed under the conditions which cause isomerism.

Granted that we are dealing with cis-trans isomerism in this field, the possible scope of research still appears to be very extended. If all the double bonds in such a molecule as lycopene ($C_{40}H_{56}$), which has eleven conjugated and two isolated double bonds, could undergo cis-trans rotations, the number of possible isomers would be over a thousand. However, according to Pauling (43,59), we can place drastic limitations on this number by a consideration of the geometry of the molecule.

In such long conjugated systems of carbon-carbon double bonds, the chromophore will be essentially coplanar as a result of the partial double bond character of the single bonds. In an unbranched polyene chromophore, each double bond could exist in a cis configuration (a slight steric hindrance would be present), but, in the carotenoids, which have a chromophore consisting of dehydrogenated isoprene units, only one double bond in each C_5-unit is spatially unhindered, and very probably only this type of double bond could have a cis configuration. This steric hindrance is due to the conflict between a methyl group and a hydrogen atom when the C-methyl

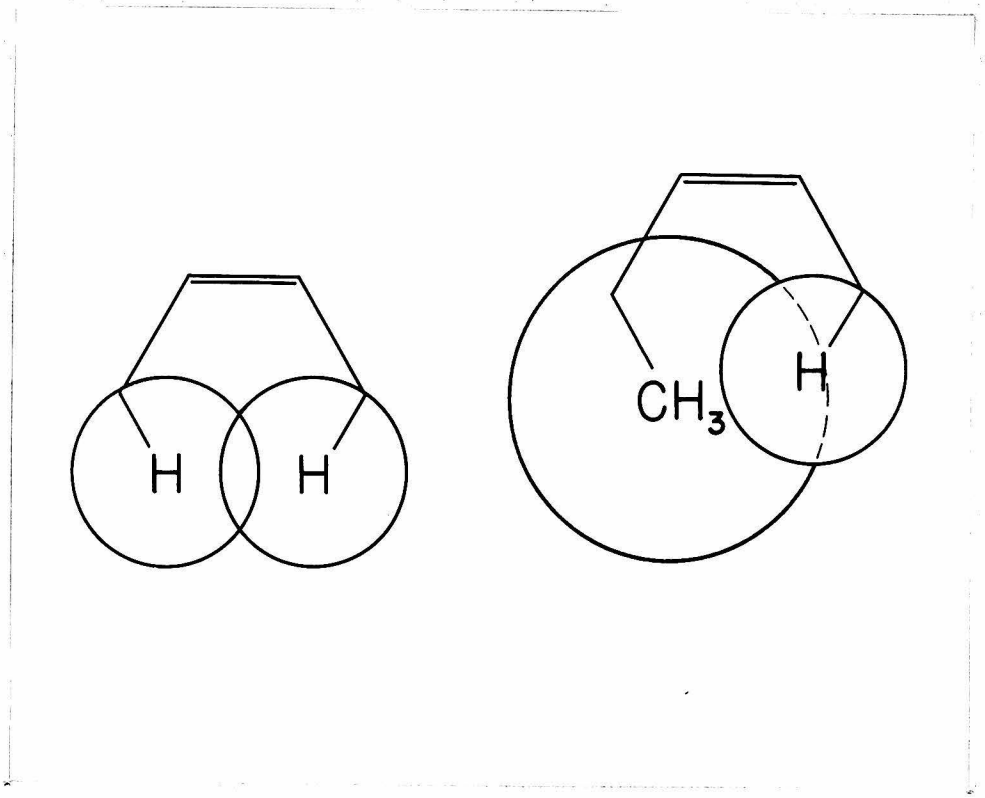


Figure 1. Drawings showing overlapping of hydrogen atoms in -CH-CR=CR-CH- and of hydrogen and methyl in -CH-CR=CR-CCH_3 with <u>cis</u> configuration (Pauling, 60).

group is adjacent to a cis double bond (Figure 1). Since this conflict is considerable, Pauling concluded that this type of double bond would be "stereochemically ineffective". Other types excluded from this discussion are those double bonds in the cyclohexene ring and also double bonds carrying a C-dimethyl group.

There are several known instances where relatively stable cis-isomers occur in spite of a marked spatial conflict (34,47,24) but these are all structures containing a shorter conjugated system in which coplanarity is more easily disturbed. Objections have been raised to this general concept of steric hindrance by Karrer (25,24) in the cases of bixin and β-methyl muconic acid ($C_7H_8O_4$), but, since the final evidence of an absolute structure determination is lacking for any stereoisomeric carotenoid, the interpretation of the results obtained in the present work will be based on the theory that double bonds of the type -CH=CH-C(CH_3) cannot assume cis configuration in such long conjugated systems.

Formulas have been given (32,60) for calculating the number of possible stereoisomers in polyenes by Kuhn and Winterstein and also Pauling. Two subclasses are recognized; the unsymmetrical and symmetrical. For unsymmetrical molecules, whose two halves are different, the number of stereoisomers, N, would be $N = 2^n$, where n is the number of stereochemically effective double bonds. For symmetrical molecules with n odd, $N = 2^{\frac{(n-1)}{2}} \cdot (2^{\frac{(n-1)}{2}} + 1)$ and for n even, $N = 2^{\frac{n}{2} - 1} \cdot (2^{\frac{n}{2}} + 1)$. In the case of lycopene, with seven stereochemically effective double bonds (Figure 2), the number of predicted isomers is seventy-two. Skeleton models of the non-hindered stereoisomeric lycopenes (50) are given in Figure 3. The distribution of lycopene stereoisomers according to the

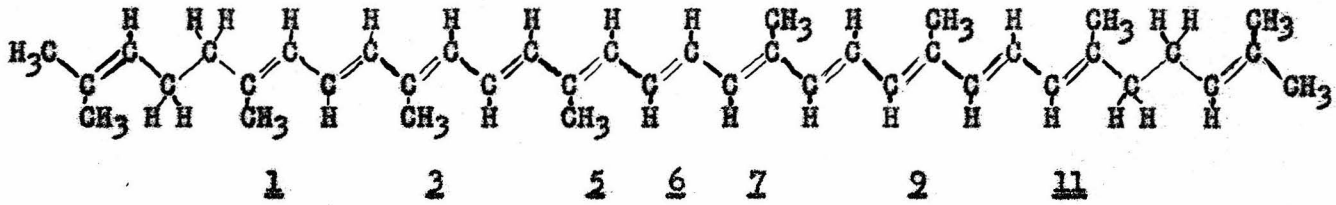


Figure 2. All-*trans* lycopene with the seven stereochemically effective double bonds numbered*

* The following nomenclature was proposed by Zechmeister and Polgar (68). Each double bond of the conjugated system will be assigned an *italicized* number to avoid confusion with the numbering of carbon atoms. The lowest number will be given to the double bond in the β-ionone ring, or, if the double bond of this ring is not part of the chromophore, to the conjugated bond nearest the β-ionone ring. In the absence of such a system, an α-ionone ring receives preference over an aliphatic terminal group. In a symmetrical molecule, evidently the numbering may be started from either end of the chromophore. Because the configurations of isomers are still unknown in most instances it is customary to employ the prefix "neo": for example, neolycopene A. The letters following A in the alphabet designate isomers with weaker adsorbability than A. If a pigment also yields stereoisomers which are adsorbed above the all-*trans* form, those with increased affinity are designated as neo T, U, V, etc. There are some inconsistencies in particular cases which arose on a historical basis. In addition, the term poly-*cis* (62) applied to a member of a given carotenoid set indicates that half or more of its stereochemically effective double bonds have the *cis* configuration. The term "pro" has been used to designate a naturally occurring neo form of a carotenoid with many *cis* double bonds, for example, prolycopene.

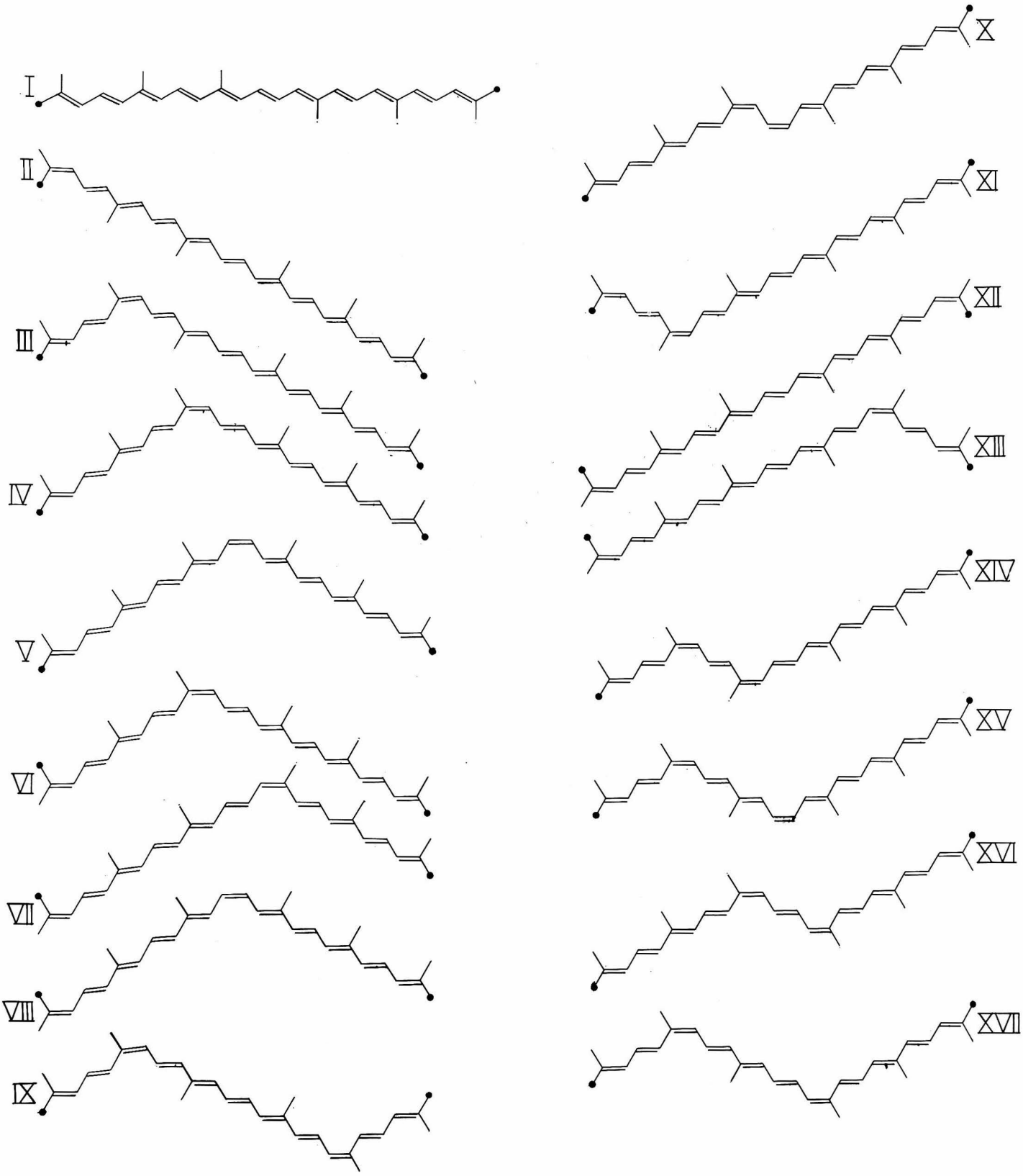
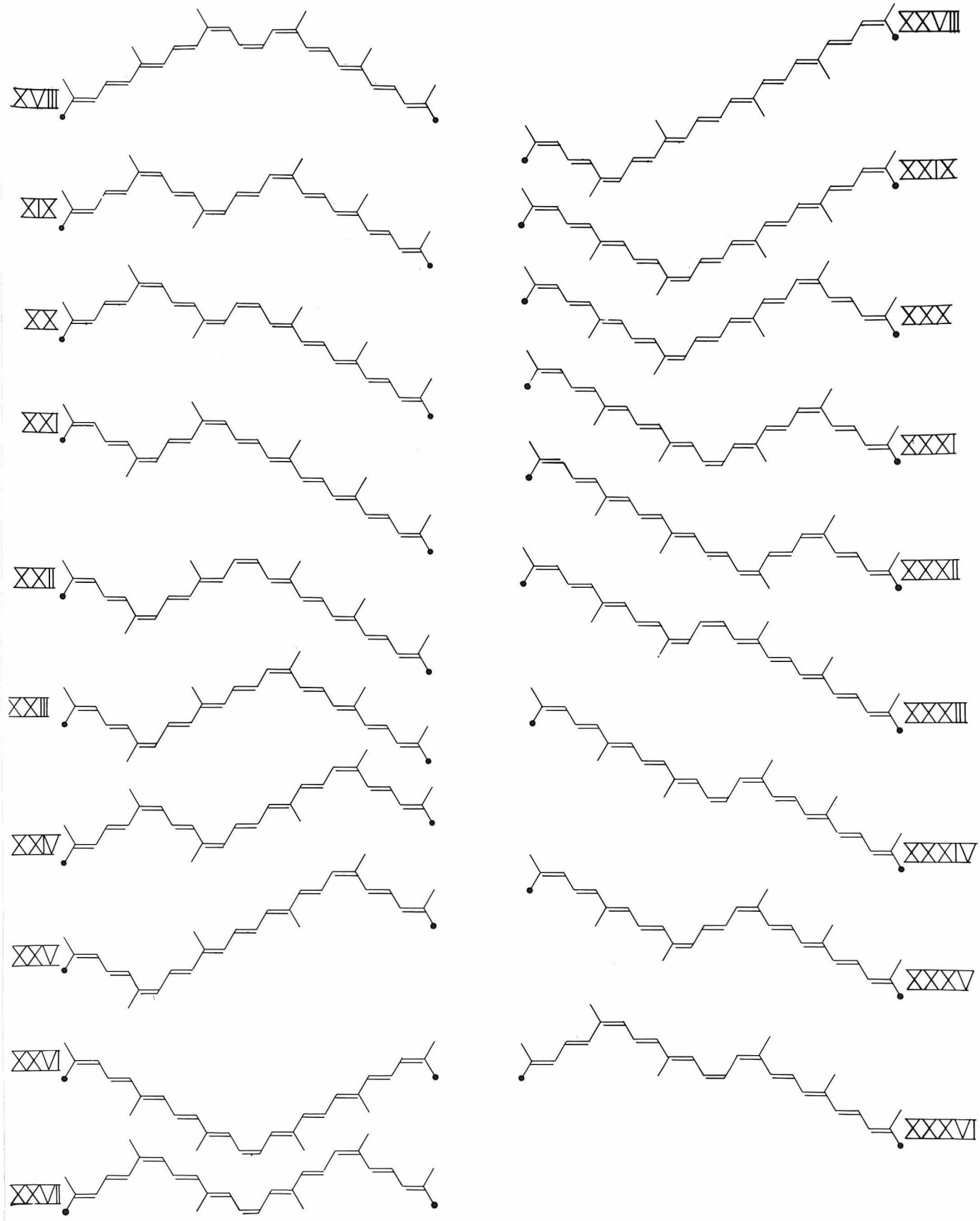


Figure 3. Skeleton models of the seventy-two sterically unhindered cis-trans isomeric forms of lycopene. The single side lines represent methyl groups; the black circles stand for the carbon skeleton of the end groups in the lycopene molecule.



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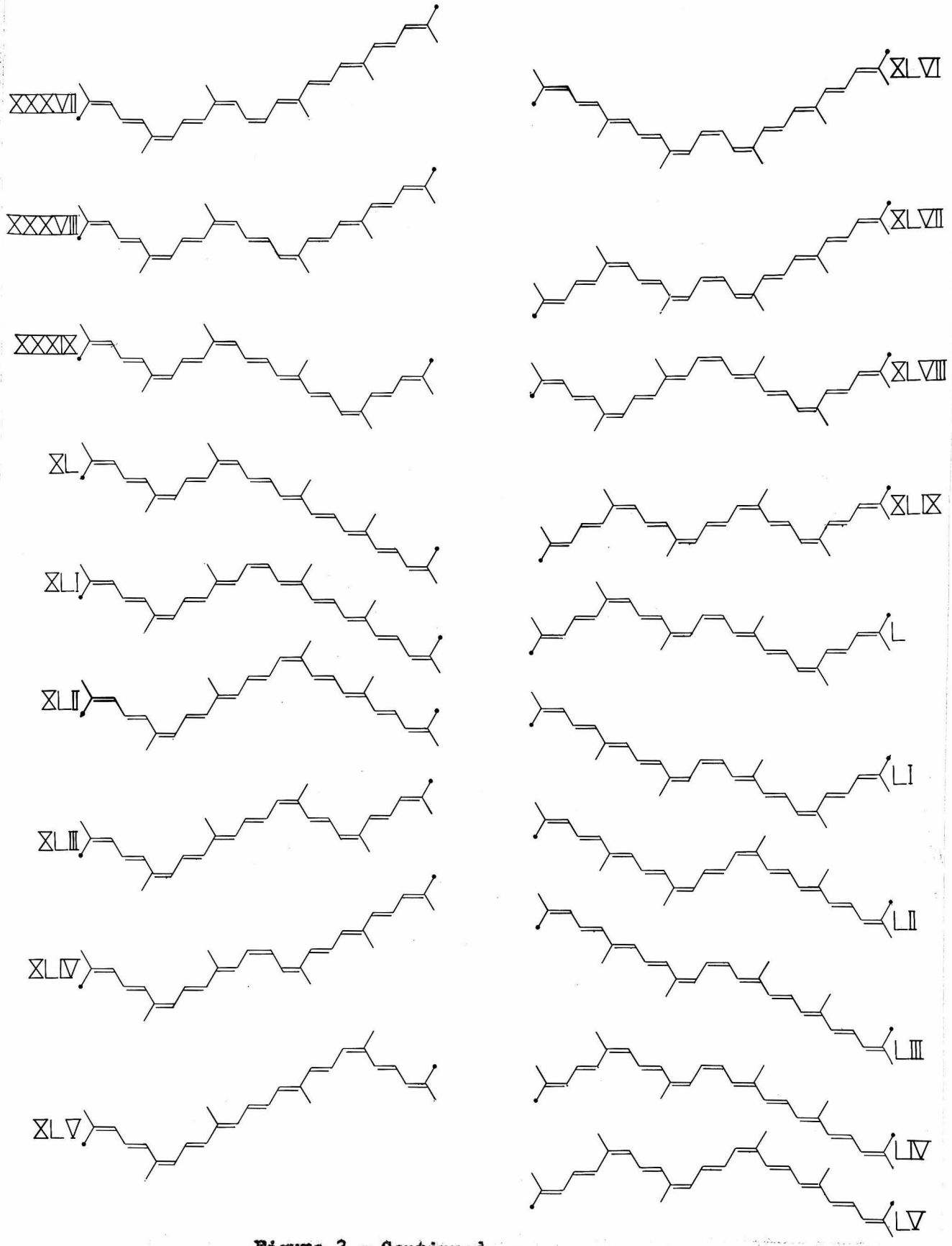

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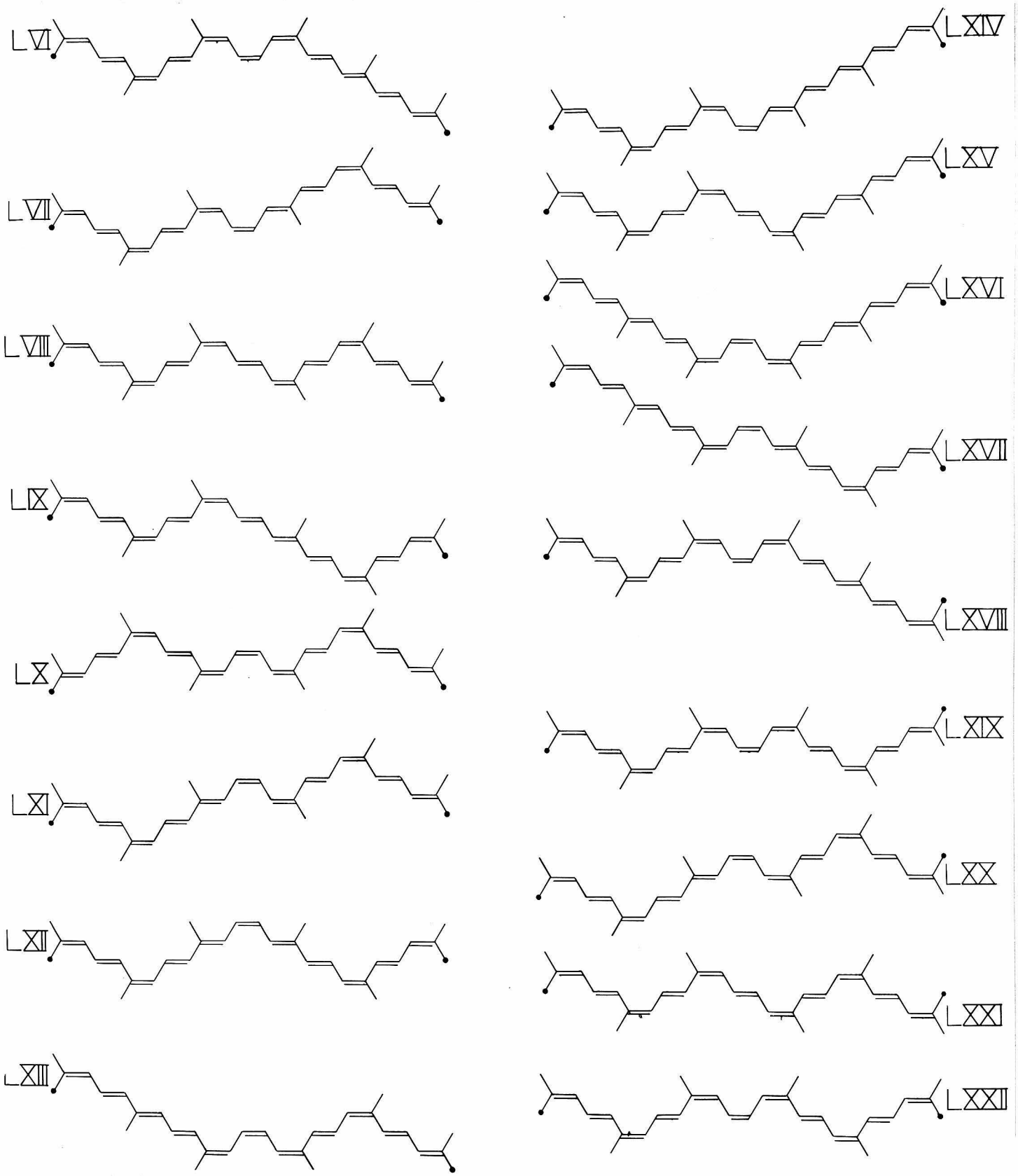

Figure 3 - Concluded

number of cis double bonds is given in Table I.

Table I

Distribution of the Sterically Unhindered Lycopene Isomers According to Number of cis Double Bonds

Type of isomer	Number of isomers
all-trans	1
mono-cis	4
di-cis	12
tri-cis	19
tetra-cis	19
penta-cis	12
hexa-cis	4
hepta-cis	1

2. Spectral Characteristics of Carotenoids

Since much of the present knowledge about the number and arrangement of cis double bonds in carotenoids is based on a study of their light absorption, a brief discussion of some theoretical and experimental results obtained by earlier authors in this field will be given (49,43,59).

That member of a carotenoid stereoisomeric set* which has the maximum light absorption in the fundamental band is believed to be the all-trans member of the set. This assumption is reasonable since the all-trans form would be the most coplanar of all possible steric forms. Even in those

* The term "Stereoisomeric set" includes all cis-trans isomers of a given carotenoid.

double bonds which are classed as stereochemically effective, the cis configuration would involve a slight spatial conflict between the hydrogen atoms on the carbon atoms adjacent to the cis bond (43); Figure 1. This conflict would involve a slight rotation out of coplanarity and interference with complete conjugation would cause the absorption maximum to shift toward shorter wave lengths. Experimental evidence supports this explanation since it is generally observed that cis isomers have their position of maximum light absorption shifted toward shorter wave lengths as compared with the all-trans form, and that the absorption bands of the all-trans member of the set shift to shorter wave lengths when stereoisomerization occurs.*

This shift in wave length of the absorption bands is the basis from which we can estimate the number of cis double bonds. It has been found experimentally, by Zechmeister et al. (49), that, in a great many carotenoid sets, the smallest decrease in the position of the longest wave length maximum (visually determined in the Zeiss spectroscope) is near 4 to 5 mu. This may very well be the effect due to a single trans cis rotation. Furthermore, values for this decrease in other cis-isomers are approximately integral multiples of this value. Small deviations can reasonably be explained by the still unexplored effect of the position of the cis bond (or bonds) in the chromophore, since a peripheral cis double bond will not be expected to have the same interference with resonance as one which is centrally located.

Earlier studies by Zechmeister and Polgár (66) of the absorption

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* One exception to this statement has been reported in the case of neovitamin A (44,37) which cannot yet be discussed in a satisfactory manner because of the scarcity of experimental data.

spectra of carotenoids showed the presence of a band in the near ultraviolet region. The wave length difference between this new peak, termed the "cis-peak", and the longest wavelength maximum is nearly constant (142 mμ) for all C_{40}-carotenoids tested so far.

A theoretical interpretation of this effect has been given by Pauling et al. (59) which permits the approximate location of cis double bonds in some members of a stereoisomeric set.

A qualitative discussion of the theory of light absorption, as applied specifically to lycopene (Pauling and co-workers) (59), has been given, and may be applied generally for any set of carotenoid stereoisomers. According to these authors we may picture a vibration of the mobile "unsaturation" electrons along the conjugated system of double bonds, and the following classical modes of vibration may be correlated with certain bands in the absorption spectra.

a) The electrons concentrate first near one end then near the other end of the conjugated system. This mode of vibration is correlated with the fundamental absorption band.

b) The electrons move from the two ends of the conjugated system toward the middle and from the middle toward the two ends. This oscillation of charge would correspond to the "cis-peak" region or first overtone.

c) The electrons concentrate alternately in the first and third and then the second and fourth quarters of the conjugated system. This mode of vibration would correspond to the second overtone absorption band.

The intensity of an absorption band is proportional to the square of the dipole moment and, thus, essentially to the square of the length

of the conjugated system (43,38,39). Since the molecules of cis isomers have a shorter distance between the ends of the chromophore than the all-trans form, the intensity of the fundamental band of the cis-isomer should be less than that of the all-trans member of the set. The decrease in intensity of the fundamental band of a cis-isomer was noted by Zechmeister and Tuzson (78,79) for neolycopene A, and a theoretical interpretation of this effect was subsequently given by Pauling (43).

The nature of the vibration which gives rise to the "cis-peak" is such that if the chromophore is symmetrical about a center, there is no dipole moment and thus no absorption in the region of the first overtone. All stereoisomers without this center of symmetry should have some light absorption in this region, but the intensity of this band would be of appreciable magnitude only for those, such as 6-mono-cis-lycopene, which have a bend near the middle of the chromophore. The intensity of the cis-peak is estimated to be roughly proportional to the square of the distance between the center of the conjugated system and the mid-point of the straight line between its two ends (Mulliken 38, 39; Pauling 59).

A summary of the stereochemical diagnosis (49) of a given carotenoid can be based, to a considerable extent, on the observed spectral data as demonstrated in Table II.

Table II

Character of the Extinction Curves for Some Types of Carotenoid Stereoisomers

Stereoisomeric type	Shape of the extinction curve in the *cis*-peak region	Change of extinction after Iodine Catalysis	
		In the fundamental band	In the *cis*-peak region
all-*trans*	flat	decrease	increase
central mono-*cis*	peak	increase	decrease
poly-*cis*	flat	large increase	increase

B. POLY-*Cis*-LYCOPENES OCCURRING IN THE FRUIT OF *PYRACANTHA*

It was discovered earlier by several investigators (Went, Zechmeister, LeRosen, Schroeder, Escue; 60,56,76,54,75,33) that several plant materials contained prolycopene and pro- γ-carotene, and that these two pigments are poly-*cis* isomers of the ordinary all-*trans*-lycopene and all-*trans*-γ-carotene. The presence of several other poly-*cis* isomers of lycopene was also indicated by the spectral data of some pigments observed on the chromatographic column. The isolation of some of these new *cis*-isomers is described in the following section.

1. Composition of the Pyracantha pigment

The main bulk of the carotenoids in the ripe berries of *Pyracantha angustifolia* Schneid. consists of hydrocarbons (76) including some members of the α-carotene, β-carotene, γ-carotene and lycopene sets (Table III), as well as the colorless polyene, phytofluene (72). In addition to some stereoisomeric lycopenes and γ-carotenes, a well known isomer of β-carotene, neo- β-carotene U, was always observed on the chromatographic column. With reference to the latter pigment, it must be stressed that if a stereoisomer is easily produced from the all-*trans* molecule by methods similar to those used in the isolation process, then a natural occurrence of this isomer should not be claimed. However, another crystallizable *cis* pigment, termed neo- γ-carotene P, was observed as a constituent of stereoisomeric mixtures as obtained from all-trans- γ-carotene.

Table III

List of some Polyene Hydrocarbons
Isolated from Pyracantha Berries

(In the order of decreasing adsorption affinities on calcium hydroxide; petroleum ether, 0 to 8% acetone. The figures denote amounts isolated per 1 kg. of fresh berries).

All-trans-lycopene	14 mg.
Poly-cis-lycopene I	3
Poly-cis-lycopene II	0.8
Poly-cis-lycopene III	1.2
All-trans- γ -carotene	3
Neo- γ-carotene P	3
Prolycopene	46
Poly-cis-lycopene IV	about 0.05
Poly-cis-lycopene V	about 0.05
Poly-cis-lycopene VI	about 0.05
Pro- γ-carotene	13

2. Some New Stereoisomers of Lycopene

As listed in Table III, six new isomers were found and termed poly-cis-lycopenes I to VI in order of their decreasing adsorbabilities, in petroleum ether solution, on lime. Poly-cis-lycopenes I, II and III were isolated in the form of analytically pure crystals (Figures 24, 25). They are accepted as true constituents of Pyracantha for the following reasons: (a) When any one of them was refluxed in petroleum ether solution, under carbon dioxide for half an hour, a subsequent chromatogram

showed that only traces of other isomers were formed, and their spectral curves, after refluxing, showed no appreciable change. (b) Prolycopene, from which these three pigments might arise, as a result of stereoisomerization during the isolation processes, is also thermostable under the conditions just given and is not likely to be altered greatly under the conditions used for the isolation of the pigments. (c) No poly-cis-lycopene has ever been obtained after heating solutions of all-trans-lycopene.

The poly-cis-lycopenes IV, V, and VI were observed only in solution and their total quantity was small.

The poly-cis-lycopenes I-VI were accepted as belonging to the lycopene set on the following basis. (a) When their petroleum ether solutions are examined in the visual spectroscope, rather blurred bands or shadowed areas are evident. However, upon the addition of a drop of dilute iodine solution to the spectroscopic cell, the bands of the stereoisomeric lycopene equilibrium mixture (502, 471, 441.5 mu) appear almost instantly. There is a great increase in the intensity and sharpness of the bands. (b) Each of these stereoisomers was catalyzed with iodine and chromatographed. The main pigment zone was separated from the others by cutting and a mixed chromatogram test performed using an authentic sample of all-trans-lycopene. There was no separation, on the chromatographic column, of the main pigment obtained from any of the six and all-trans-lycopene. (c) The spectral curve obtained from each of the six poly-cis isomers, after iodine catalysis, proved to be practically identical with the corresponding curve of iodine catalyzed all-trans-lycopene or prolycopene

(* Figures 4-10 and Table X). (d) The analytical data (Table IV) indicate the same chemical composition.

Table IV

Carbon and Hydrogen Values Found for Some Crystalline Polyene Hydrocarbons ex Pyracantha

(Calcd. for $C_{40}H_{56}$: C, 89.48; H 10.52)

Pigment	Carbon (%)		Hydrogen (%)	
Prolycopene	89.02	89.62	10.67	10.87
Poly-cis-lycopene I	89.04**	89.11	10.56**	11.03
Poly-cis-lycopene II	89.34		10.55	
Poly-cis-lycopene III	89.76	89.49	10.59	10.58
All-trans- γ-carotene	89.20**	89.49	11.01**	10.58
Neo- γ-carotene P	89.18		10.57	
Pro- γ-carotene	89.56		10.92	

3. Spectral Characteristics of the Poly-cis-lycopenes

As mentioned earlier, it has been observed, in all stereoisomeric carotenoid sets tested so far, that each trans ⟶ cis shift causes a certain decrease in the wave length position of the maximum of the

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* In all spectral curves which follow, the term $E_{1\ cm.}^{mol.}$ is defined as equal to $\frac{1}{L \cdot c} \cdot \log \frac{I_o}{I}$ where L is the length of the solution in the light path; c is the concentration in moles per liter; and I_o and I are the initial and final light intensities (66). L is expressed in cm.

** These values have been corrected for about 1% ash.

fundamental absorption band. The smallest such decrease known (59) for a member of the stereoisomeric lycopene set is 7.5 mu, in hexane, for neolycopene A (determined in the Beckman spectrophotometer). If this value is assumed to be that which corresponds to a single trans⟶cis shift, the probable number of cis bonds can be estimated for some members of the lycopene set (Table V).

Table V

Probable Number of cis Double Bonds of Some Stereoisomeric Lycopenes, Based on Extinction Data in Hexane

(The pigments are listed in the sequence of decreasing wave lengths of $\lambda_{max.}$ and increasing number of cis double bonds probably present)

Member of the set	$\lambda_{max.}$ (mμ)	Diff. in $\lambda_{max.}$ from the all-trans form (Beckman spectrophotometer) (mμ)	$E^{mol.}_{1\ cm.}$ x 10^{-4} at $\lambda_{max.}$	Relative Extinction areas between 320 and 560 mμ	Probable Number of cis Double Bonds Present
All-trans	472-473	0	18.6	100	-
Neo A	465	7.5	12.2	82	1
Poly-cis I	444-445	28	12.3	76	4-5
Poly-cis III	444-445	28	11.3	71	4-5
Poly-cis II	441	31.5	11.4	70	4-5
Prolycopene	438	34.5	10.3	60	5-6
Poly-cis VI	433	39.5	8.1	54	6
Poly-cis V	431-432	41	9.0	52	6
Poly-cis IV	426	46.5	10.4	62	6-7

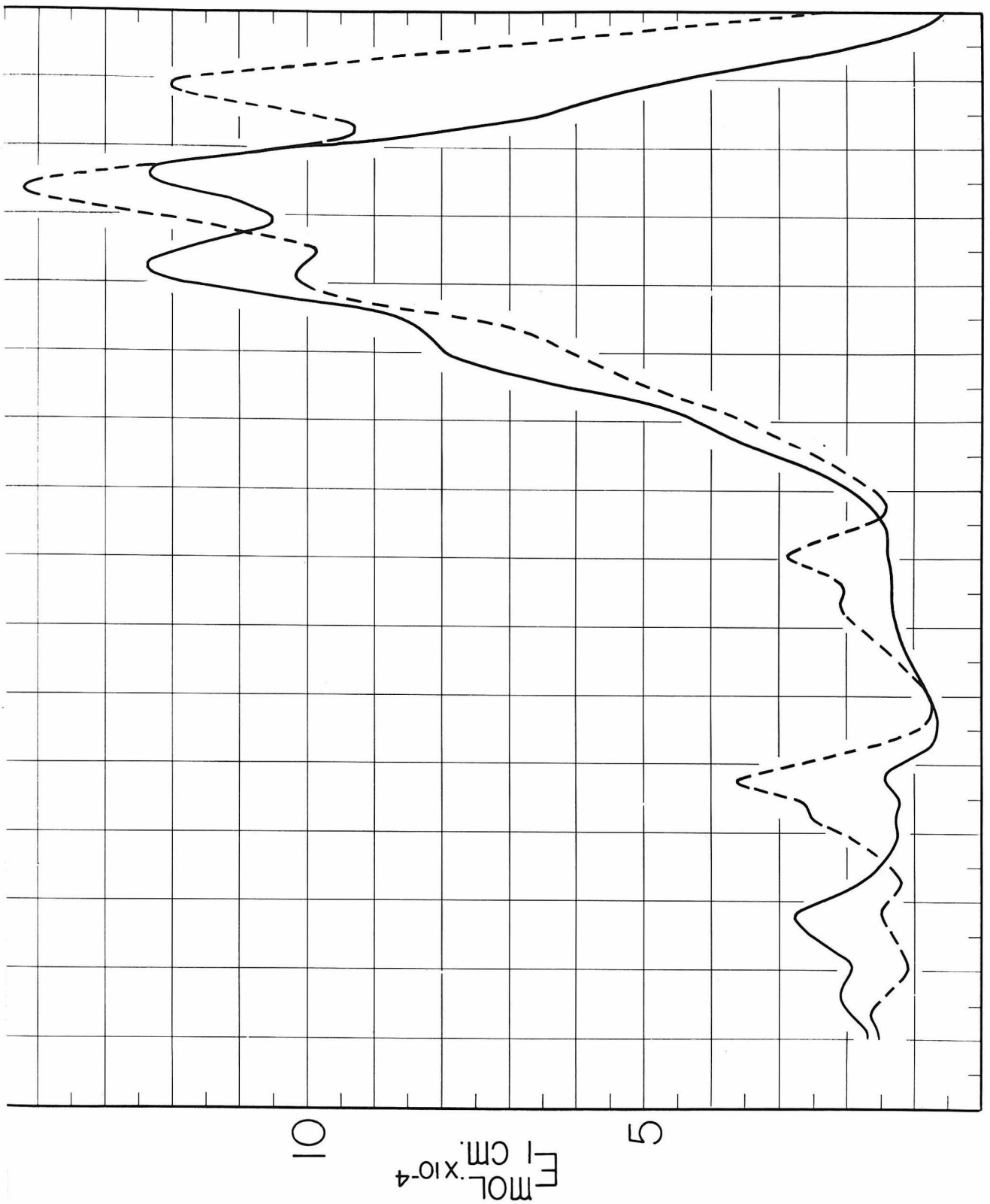

Figure 4. Molecular extinction curves of poly-cis-lycopene I in hexane: ——, fresh solution; -----, after iodine catalysis, in light.

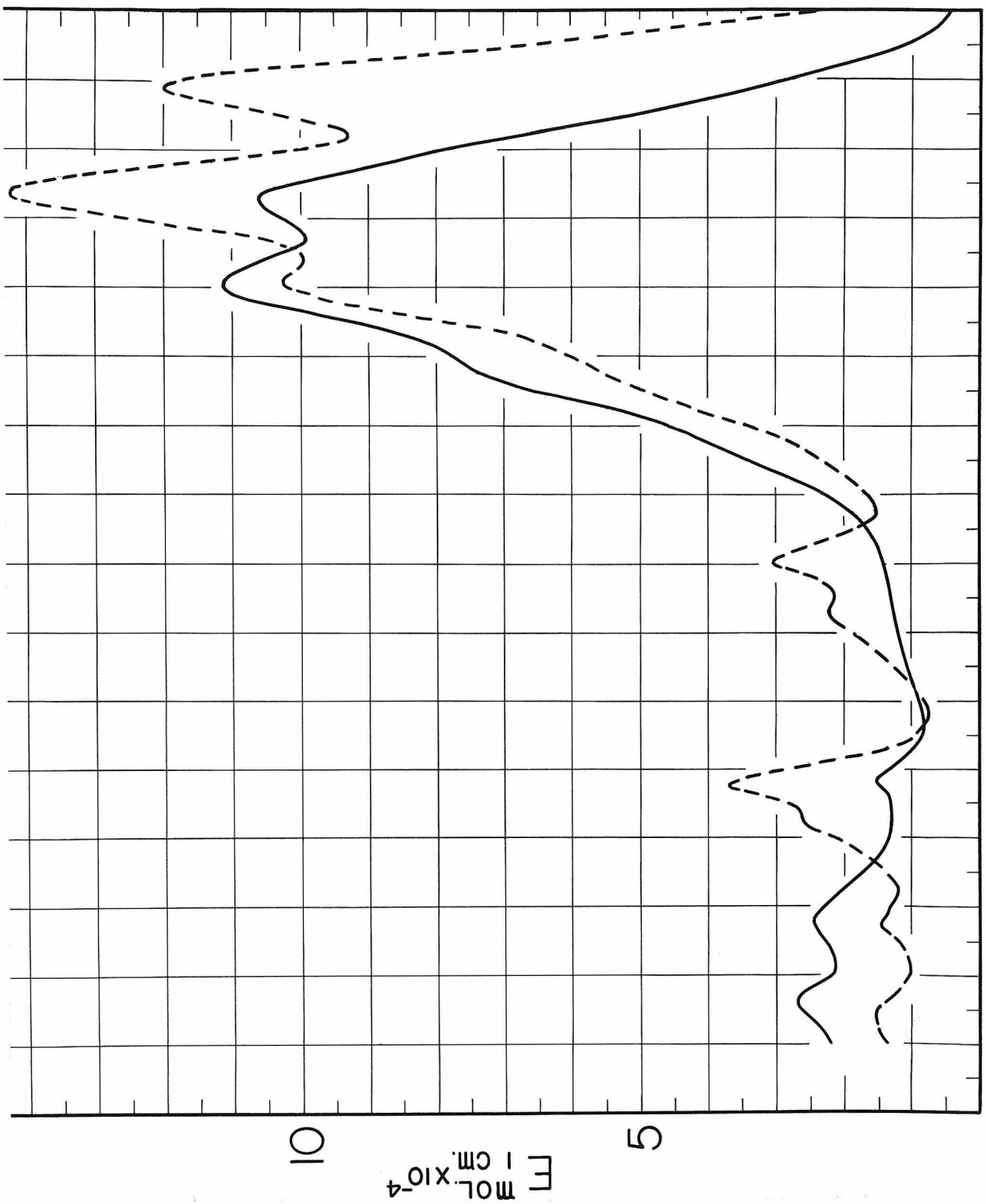

Figure 5. Molecular extinction curves of poly-cis-lycopene II in hexane: ——, fresh solution; - - -, after iodine catalysis, in light.

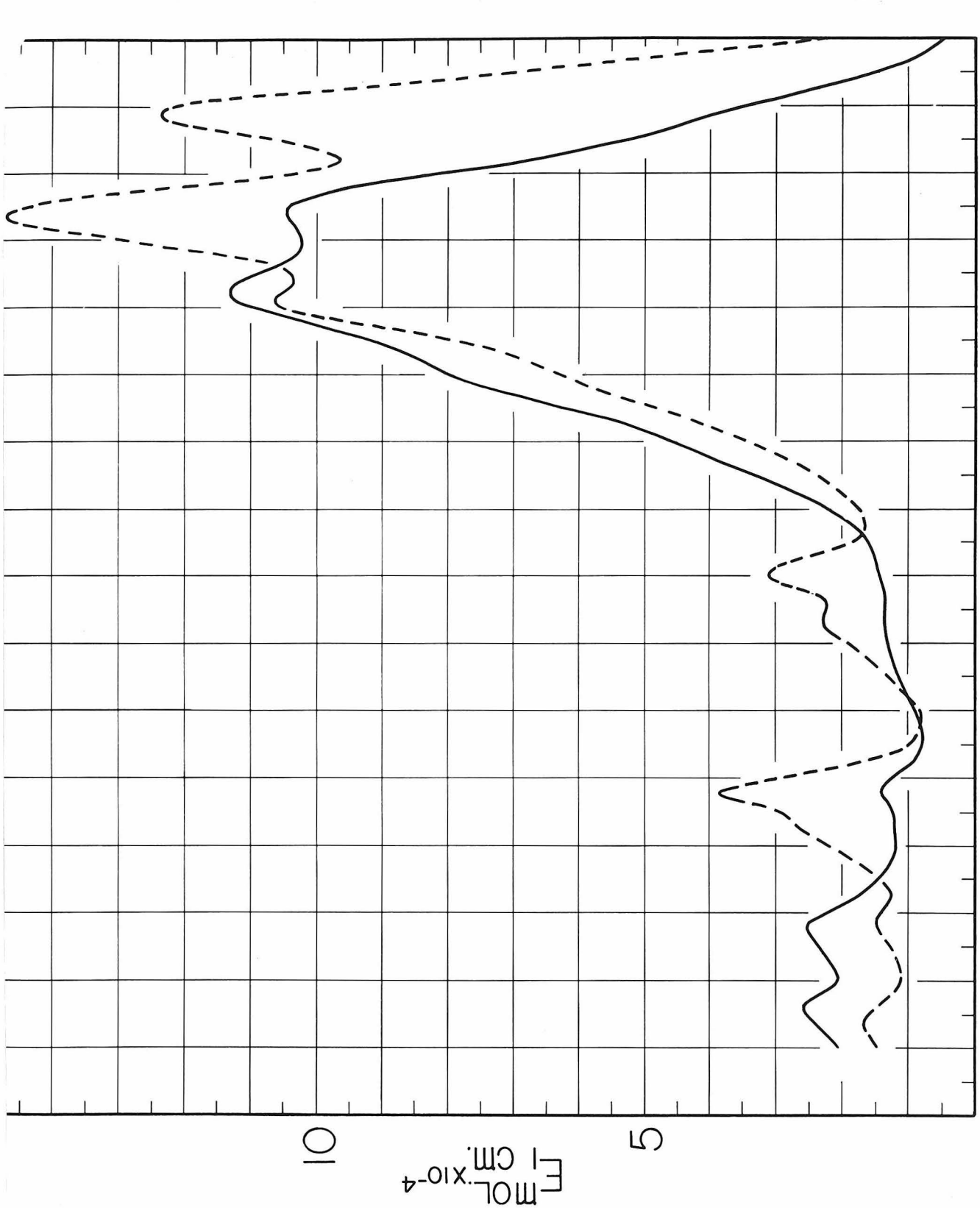

Figure 6. Molecular extinction curves of poly-cis-lycopene III in hexane: ——, fresh solution; ----, after iodine catalysis, in light.

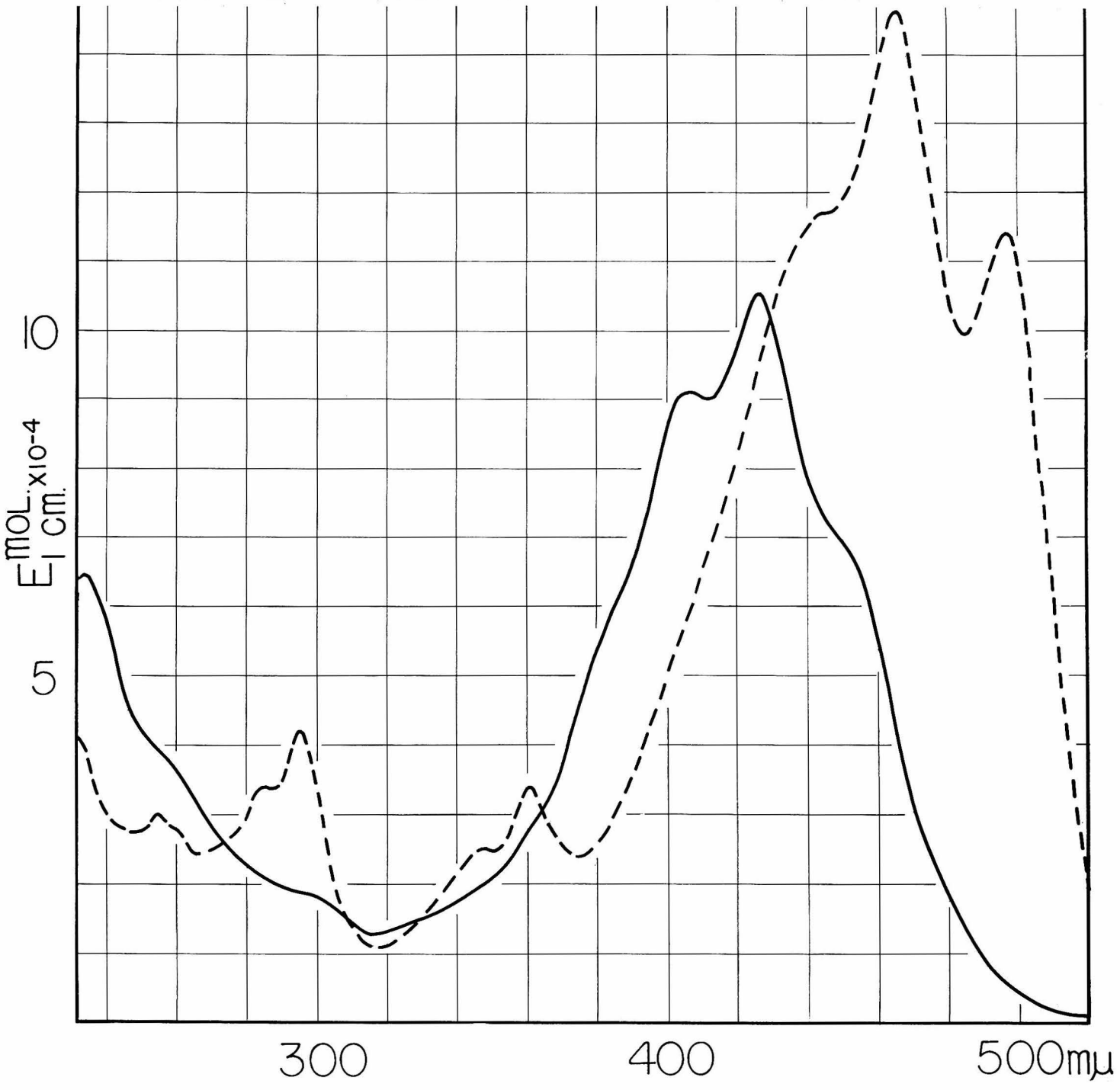

Figure 7. Molecular extinction curves of poly-<u>cis</u>-lycopene IV in hexane: ———, fresh solution; - - - - -, after iodine catalysis, in light.

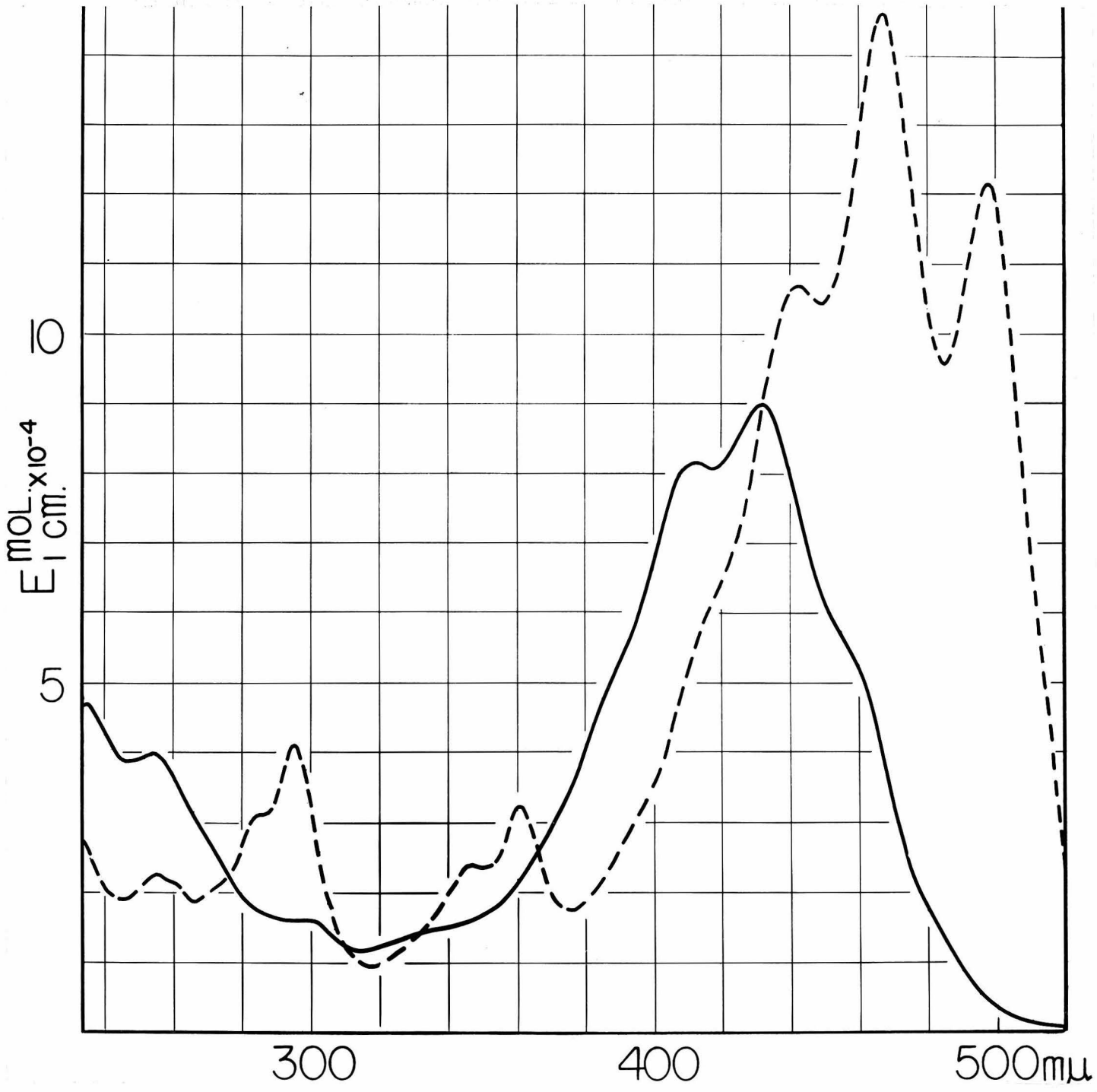

Figure 8. Molecular extinction curves of poly-cis-lycopene V in hexane: ———, fresh solution; - - - - -, after iodine catalysis, in light.

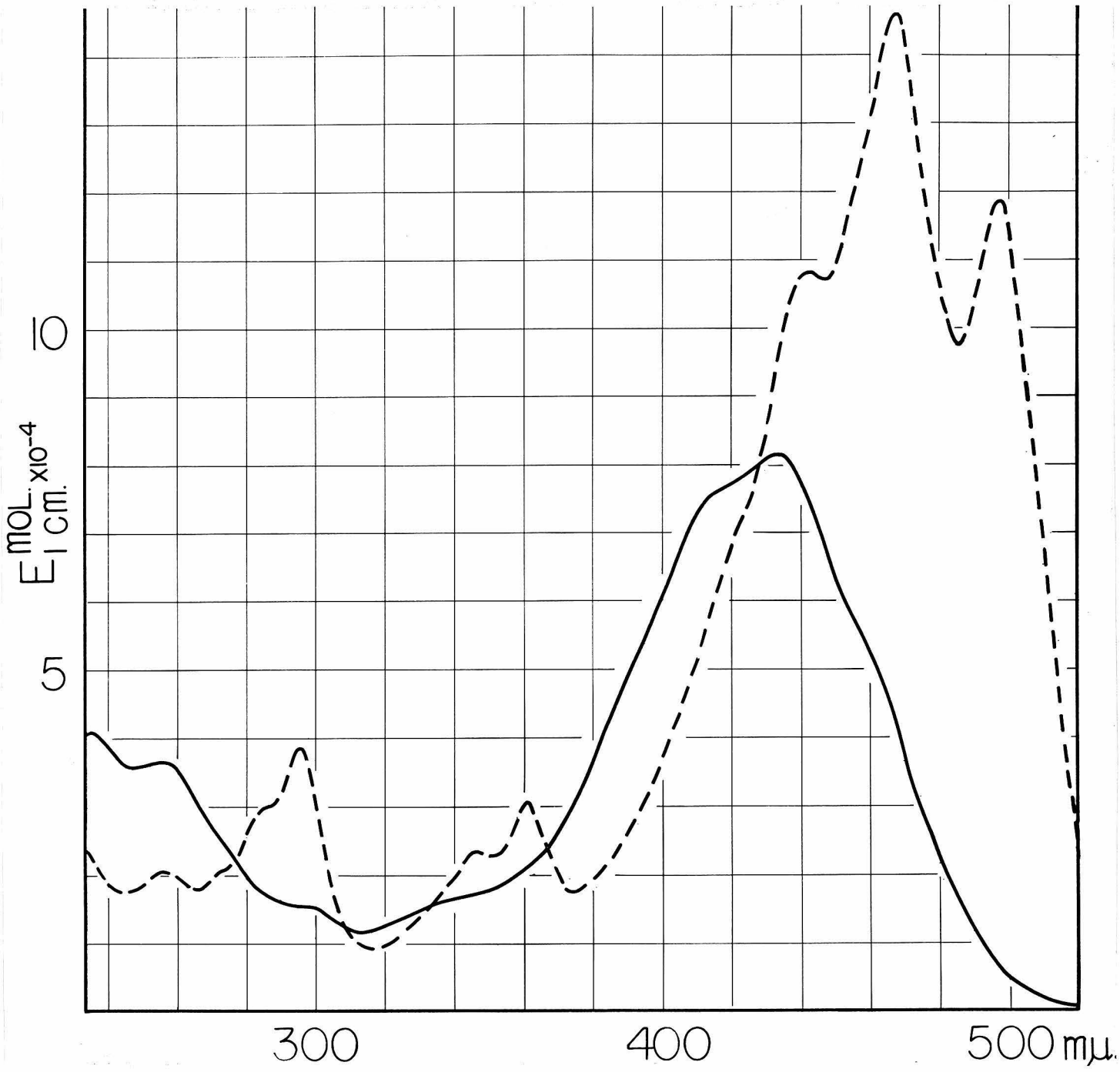

Figure 9. Molecular extinction curves of poly-cis-lycopene VI in hexane: ———, fresh solution; - - - - -, after iodine catalysis, in light.

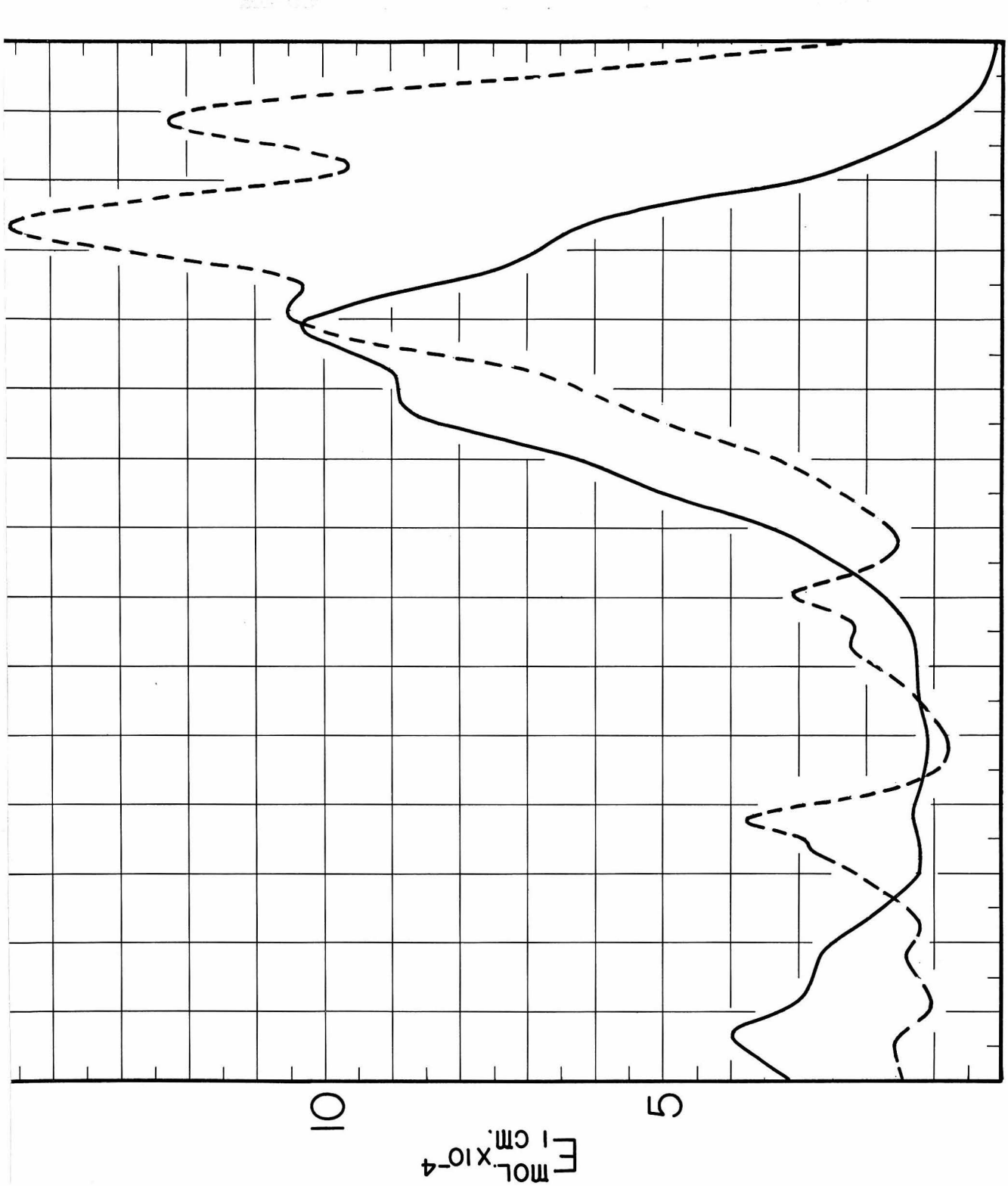

Figure 10. Molecular extinction curves of prolycopene in hexane: ——, fresh solution; - - -, after iodine catalysis, in light.

A strict proportionality between the number of cis bonds and the value of the spectral shift is not to be expected, but the estimates in Table V should be close approximations to the correct number (perhaps ± one cis double bond). The poly-cis-lycopenes from Pyracantha have four to seven cis bonds out of the seven sterically unhindered double bonds.

The intensity of the fundamental band for each of the stereoisomeric poly-cis-lycopenes is considerably less than that of the all-trans form. Adopting the suggestion made by Pauling (59), the area of the extinction curves is taken as a measure of the intensity of light absorption. The areas under the curves, from 320 to 560 mμ for each of the poly-cis-forms, were measured with a planimeter, and these values were compared to the corresponding area of all-trans-lycopene (Table V).

In the theory developed to explain the spectral characteristics of the lycopene set (59), it was pointed out that all isomers with a vertical plane of symmetry have a distance between the ends of the conjugated system smaller than the all-trans form by a factor of cos α, with $\alpha = 27^{\circ}\ 22'$ if the carbon-carbon bond angle along the chain is $125^{\circ}\ 16'$. Since the intensity of light absorption is proportional to the square of the dipole moment and, thus, to the square of the distance between the two ends of the conjugated system, all isomers of this type should have intensities approximately 80 per cent as great as the all-trans- form ($\cos^2 27^{\circ}\ 22' = \sim 0.8$). All other isomers would be expected to show intensities between 80 and 100 per cent of that of all-trans-lycopene (59). We notice from Table V that the smallest area for any of the poly-cis forms amounts to about 50 per cent of the area covered by the curve of all-trans-lycopene, and that all of the poly-cis forms have areas less than 80 per cent of the all-trans form.

The lesser intensity of the fundamental band of poly-cis-lycopenes becomes evident on simple inspection of either a solution or adsorbate. All-trans-lycopene solutions are intensely red while a stereoisomer such as poly-cis-lycopene VI appears to be yellow at similar concentrations.

The decrease in fine structure of the fundamental band of an all-trans carotenoid is particularly conspicuous when the number of cis double bonds increases. Steric forms with one peripherally located cis double bond such as neo- α -carotene U or neo- β -carotene U show a moderately decreased extinction without much alteration in the relative heights of the peaks which defines the degree of fine structure. A central mono-cis isomer such as neolycopene A shows somewhat more of a change. The ratios of the extinction values at the two longest wave length maxima and the intermediate minimum are 1.45 : 1 : 1.77 for neolycopene A; while for all-trans-lycopene the corresponding values are 1.72 : 1 : 1.86; and for poly-cis lycopene II, 1.07 : 1 : 1.12. In the spectral curve of poly-cis-lycopene VI (Figure 9) there is scarcely an indication of fine structure.

An examination of the models in Figure 3 shows that most of the isomers with three or more cis double bonds are characterized by an over-all linear shape of their molecules. Such a rod-like shape is also characteristic for those isomers which have one peripherally located cis double bond or two adjacent cis double bonds. No cis-peak (in the lycopene set, at around 360 mμ) is to be expected for isomers of this type. The same statement is valid for all seven poly-cis-lycopenes known at the present time (including prolycopene). No cis peak appears in the region between 320 and 380 mμ in any instance for these steric forms.

The region of the second overtone, which is located near 295 mu in the case of all-trans-lycopene, is more difficult to interpret in the curves of its poly-cis isomers. If the second overtone band has shifted to shorter wavelength, as the fundamental band has shifted, then it may be represented by the peaks near 255 or 235 mμ. These peaks show a decrease in height (compared to the 295 mμ peak of the all-trans form) which parallels the decrease in height of the fundamental band of the poly-cis forms as compared to that of all-trans-lycopene.

4. Adsorption Characteristics of the Poly-cis-lycopenes

Although the exact relationship between adsorption affinities and stereochemical configuration is unknown, some general statements can be made. It was shown earlier (78,79) that the mono-cis isomer, neolycopene A, had a moderate decrease in adsorption affinity on a lime column as compared to the all-trans compound, in ligroin solution. All poly-cis isomers known at the present time show much less adsorption affinity than the corresponding all-trans form; furthermore the individual differences between the poly-cis forms are much smaller than the difference between the all-trans form and any poly-cis compound. One can say, as an approximation, that the adsorbabilities decrease in the same order as the extinction values of the fundamental band. Figure 11 shows the position and shape of the top section of the respective fundamental bands and demonstrates at the same time the approximate adsorption sequence. The effect of the accumulation of cis bonds on the adsorption affinity is so great that some poly-cis-lycopenes are adsorbed below all-trans-γ-carotene.

5. Cis isomers in the γ-carotene set

In contrast to the large number of lycopene isomers in Pyracantha, the γ-carotene set seems to be represented only by three of its members;

all-trans- γ-carotene, pro- γ-carotene, and a new isomer termed neo- γ-carotene P.

The absorption curve of neo- γ-carotene P (Figure 12) indicates that it is a mono-cis isomer; and since no marked cis-peak is present the cis bond is probably located near the end of the chromophore. Its slightly decreased adsorption affinity on the lime column as compared to the all-trans form is in agreement with the behavior of corresponding lycopene isomers. Neo- γ-carotene U, which was described earlier (69), is adsorbed above the all-trans isomer and is presumably also a peripheral mono-cis isomer. Since neo- α-carotene U and neo- β-carotene U are both adsorbed above their all-trans forms, this increased adsorption affinity of some steric forms, having peripheral cis double bonds, may be related to the presence of ring structures at the ends of the carotene chromophores. The possibility arises that neo- γ-carotene U has the cis bond near the hydroaromatic ring while, in neo- γ-carotene P, the cis double bond could be located near the aliphatic end of the molecule. This would be in accordance with observations in lycopene, which has a completely open chain structure and, all of whose known stereoisomers show decreased adsorption affinity in comparison to the all-trans form.

The extinction curves of all-trans- γ-carotene (Figure 13) and pro- γ-carotene (Figure 14) agree in all main features with curves published earlier (59). Some results of bioassays carried out with all-trans- γ-carotene and its two stereoisomers, pro- γ-carotene and neo- γ-carotene P are given in Part I, Section D.

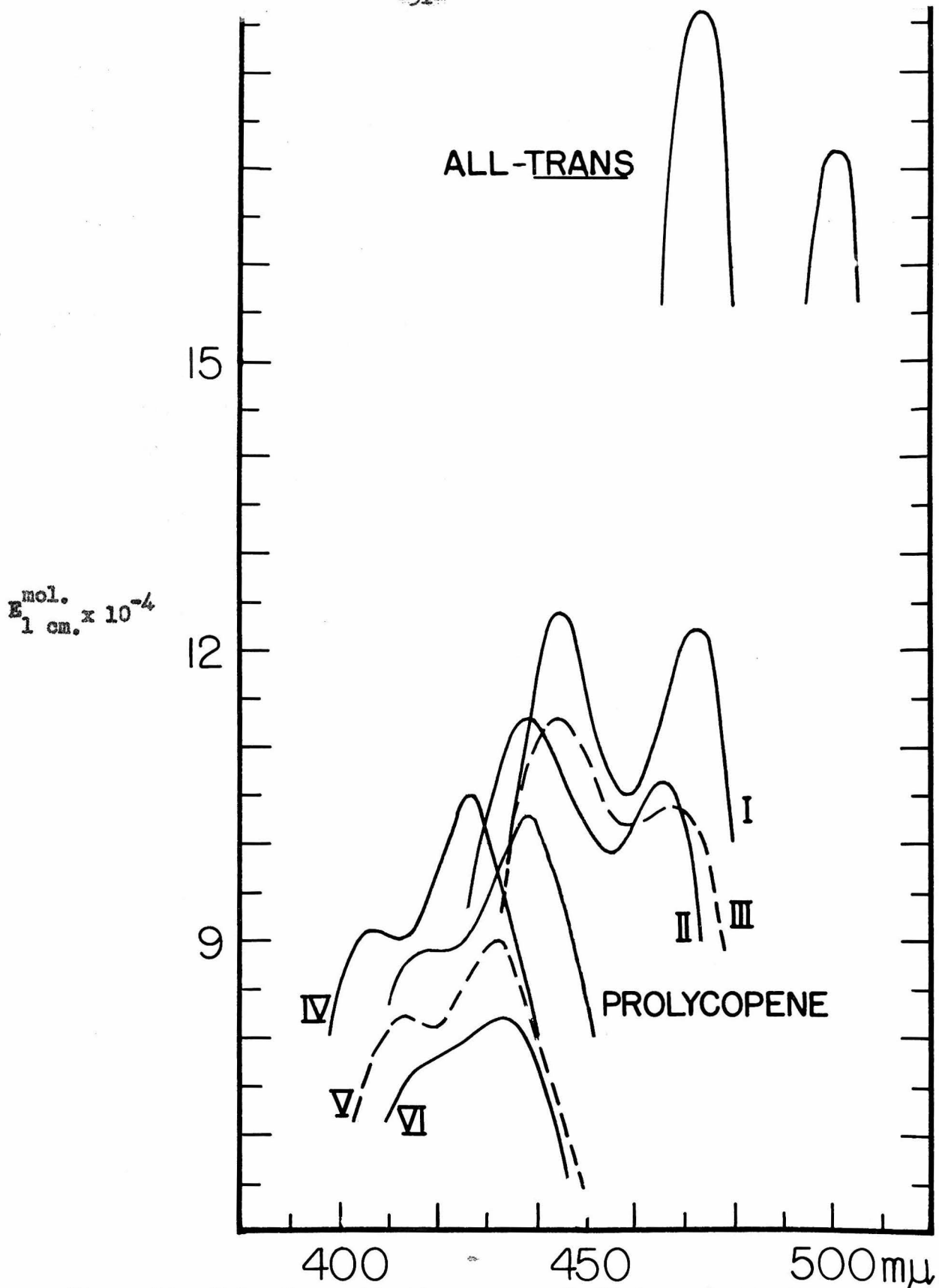

Figure 11. Molecular extinction curves of all-trans-lycopene, prolycopene and poly-cis-lycopenes I - VI at their main maxima, in hexane.

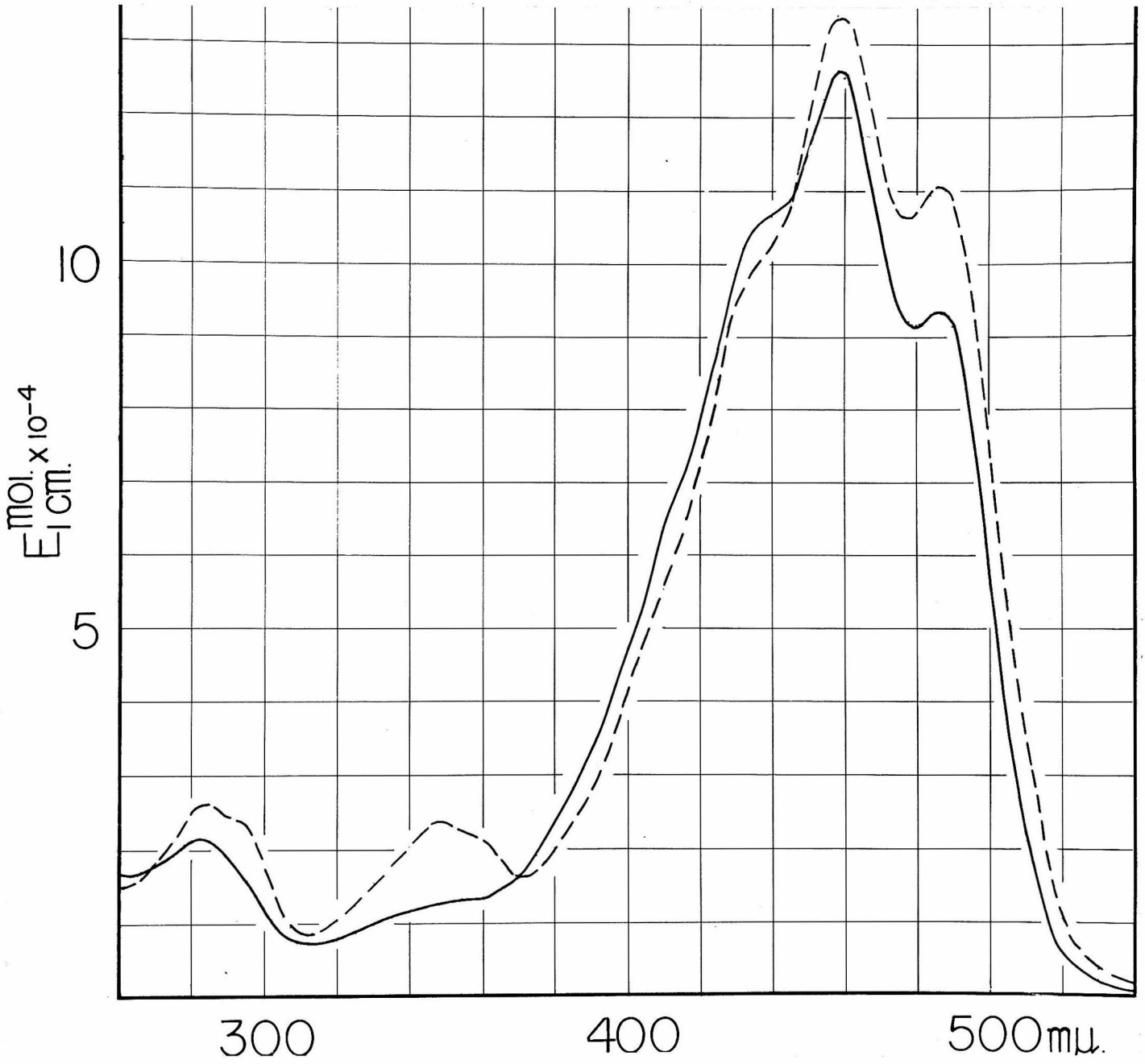

Figure 12. Molecular extinction curves of neo-γ-carotene P in hexane: ——, fresh solution; - - - - -, after iodine catalysis, in light.

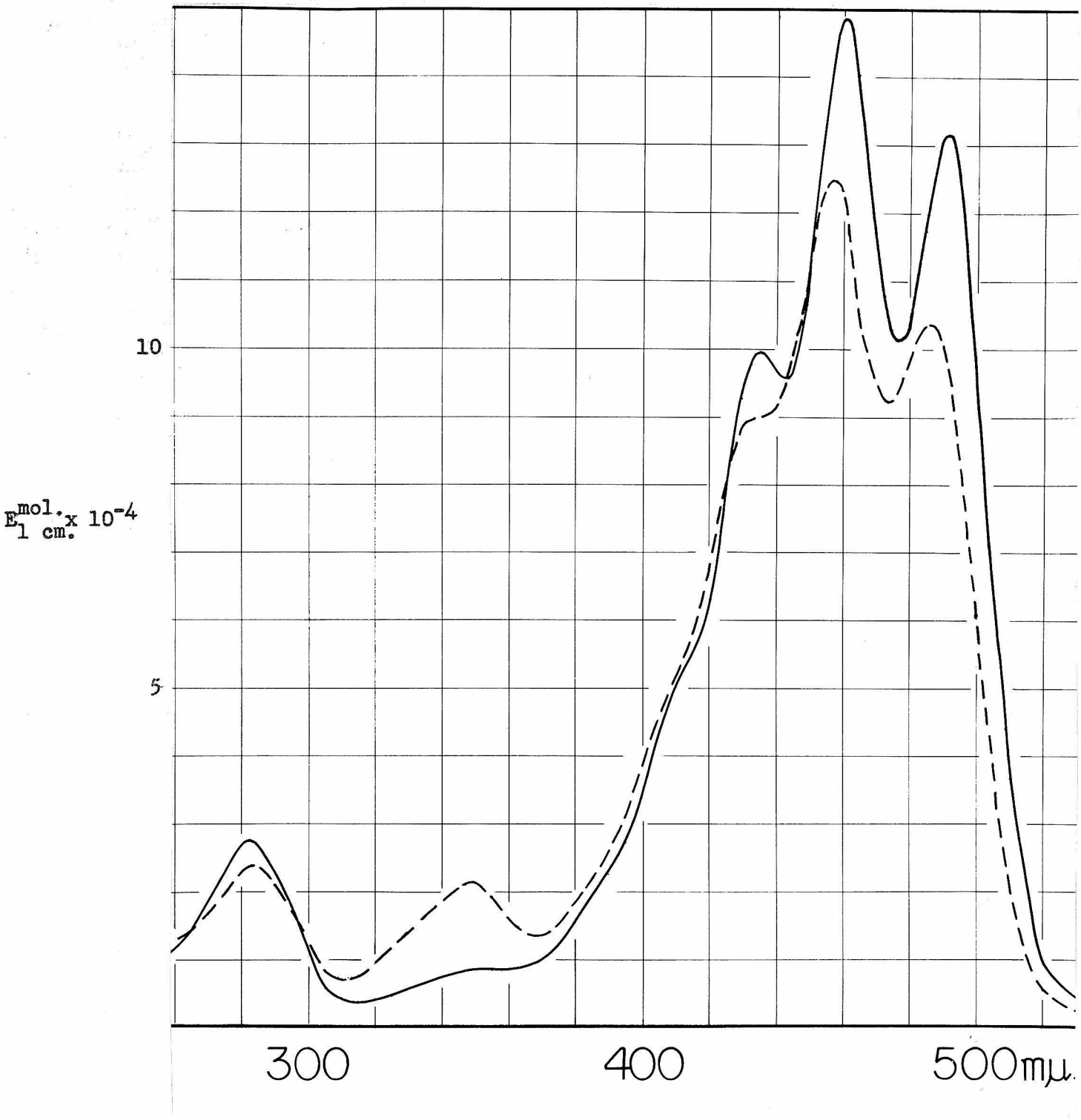

Figure 13. Molecular extinction curves of all-trans- γ -carotene in hexane: ———, fresh solution; - - - - -, after iodine catalysis, in light.

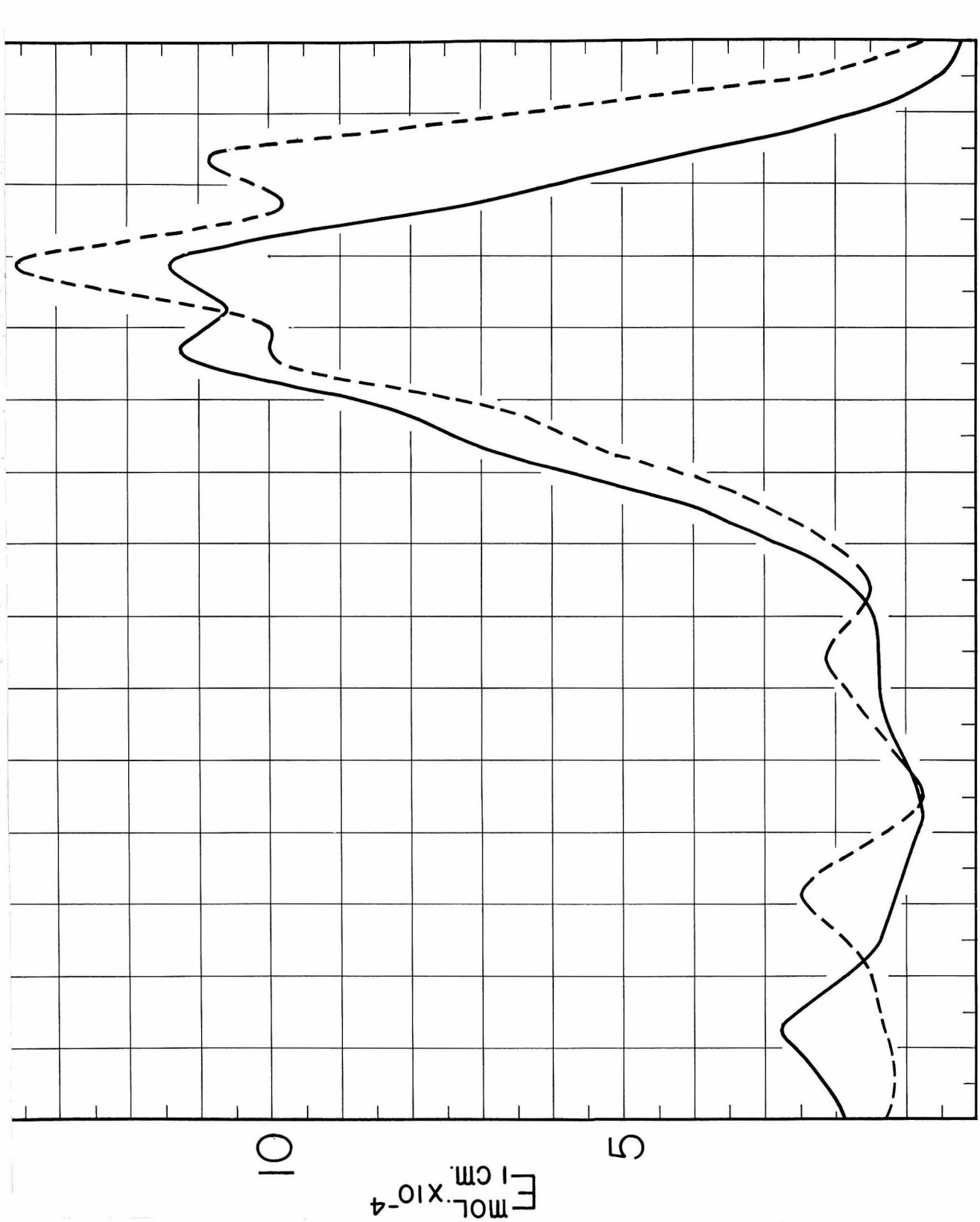

Figure 14. Molecular extinction curves of pro-γ-carotene in hexane: ——, fresh solution; ----, after iodine catalysis, in light.

C. POLY-*Cis*-LYCOPENES OCCURRING IN THE "TANGERINE" TOMATO

At first investigation of the pigments in the "tangerine" variety of tomato (56) had indicated the presence of a considerable number of stereoisomeric lycopenes, but that early study had to be confined to the main pigment, prolycopene.

An effort to isolate some of these minor isomers has now succeeded. In addition to the main pigment, prolycopene, two other poly-*cis*-lycopenes were isolated in the form of analytically pure crystals. These pigments proved to be identical with the poly-cis-lycopenes I and III (*ex* Pyracantha). The isolation of these two forms from a different plant material ("tangerine" tomato) is a confirmation of the earlier data obtained with Pyracantha, especially since the spectroscopic characteristics of these two compounds were found to be the same in samples from the two source materials.

D. THE PROVITAMIN A POTENCY OF SOME STEREOISOMERIC γ-CAROTENES

1. Previous Work on Stereoisomeric Provitamins A

The relation between spatial configuration and provitamin A activity of some carotenoids has been the object of several earlier studies. Qualitative work was carried out first by Gillam and co-workers (16,17) for an unidentified stereoisomer of β-carotene. They concluded that, in the rat, the vitamin A potency of the new steric form was of the same order of magnitude as that of all-trans- β-carotene itself. Kemmerer and Fraps (14,27,28) later tested the same stereoisomer and found a potency one-half that of β-carotene.

A series of investigations by Deuel, Zechmeister and group (6,7,8, 9,10,11) have covered a number of members in the stereoisomeric α-, β-, and γ-carotene sets as well as in the cryptoxanthin set.

The change in provitamin A potency when an all-trans carotenoid undergoes one or more trans ⟶ cis rearrangements is a general phenomenon. If complete information were available on the bio-activity of the possible stereoisomers of vitamin A*, a more definite choice could be made between possible reasons for this change in potency. In accordance with the theory developed (43) about the number of stereochemically effective double bonds, the probable number of (non-hindered) steric forms of vitamin A would be four. So far, only one cis-trans isomer of vitamin A (neovitamin A) has been reported (44), and a preliminary test indicated that its potency in the rat is roughly equivalent to that of vitamin A itself.

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* "Vitamin A" in this thesis should be interpreted as referring to vitamin A_1.

Among the possible reasons for a biopotency differing from that of the all-trans carotenoid are:

a) The degree of steric stability and chemical resistance of the provitamin A or vitamin A isomers in the body.

b) Solubility or adsorption differences.

c) Specificity of enzyme systems for particular configurations.

d) Various requirements for different species of animals.

Some values obtained in this laboratory for biopotencies of isomeric provitamins A in the rat are given in Table VI.

Table VI

Relative Provitamin A Activities of Some Carotenoids in the Rat

Substance	Relative potency (%)	Literature Reference
All-trans-β-carotene	100	-
Neo-β-carotene U	38	(8)
Neo-β-carotene B	53	(6)
All-trans-α-carotene	53	(11)
Neo-α-carotene U	13	(11)
Neo-α-carotene B	16	(6)
All-trans-γ-carotene	28	(7)
Neo-γ-carotenes (mixture)	16	this Thesis
Pro-γ-carotene	44	(7)
Neo-γ-carotene P	19	this Thesis
All-trans-cryptoxanthin	57	(9)
Neocryptoxanthin A	42	(10)

2. Configuration and Bio-potency in the γ-carotene Set

Kuhn and Brockmann (29) first reported the provitamin A activity of a γ-carotene sample isolated from commercial carotene. Their experiments with rats (30) gave a value of approximately 50% as compared to β-carotene.

An investigation by Deuel, Zechmeister et al. (7) using γ-carotene ex Mimulus longiflorus gave values of 28% that of β-carotene and, in the same experiment, pro- γ-carotene had an activity of 44%. A similar investigation was made with γ-carotene and neo- γ-carotene P which were isolated from Pyracantha (62), and with mixed neo- γ-carotenes prepared by refluxing solutions of γ-carotene. The activities found (Table VI) were all lower than that of all-trans- γ-carotene, while the potency of all-trans γ-carotene itself was close (26% versus 28%) to that reported earlier.

Since, of all pigments studied, pro- γ-carotene was the only one whose activity seems to exceed that of all-trans- γ-carotene, it was desirable to clarify some doubts about the relation between pro- γ-carotene and the all-trans- γ-carotene as used in earlier tests.

All-trans- γ-carotene isolated from Mimulus agreed in most properties with other authentic samples of -carotene. However, there was a great variation in the reported melting points (131 to 178°) and all efforts to raise the melting point above 150° failed (74). Most samples gave values ranging from 131 to 150°. The same behavior was observed with samples of γ-carotene isolated from several different sources (62,74). Since it is conceivable that the pro- γ-carotene and the all-trans- γ-carotene used in the biological tests belonged to two different stereoisomeric sets, (because of slight, still unrevealed structural differences), it was decided to prepare an all-trans- γ-carotene sample from pro-

γ-carotene by iodine catalysis, and to run comparative bioassay on the two substances.

Little quantitative information had been available concerning the utilization of carotenoids in the chick. T. K. With (48) carried out the first extensive studies on the provitamin A effect of carotenoid pigments in the chick. He concluded that cryptoxanthin is a more potent provitamin A for the chick than β-carotene. Some doubt exists, however, of the validity of this statement, since With did not use pure cryptoxanthin, but based his results on feeding yellow corn, etc. Recently, Baumann and his group (22) reported feeding experiments which showed that pure cryptoxanthin is even inferior in potency to β-carotene.

Considering the importance of the question, whether, in principle, a poly-cis carotenoid may have a biopotency as great as its all-trans form, comparative bioassays were also carried out in the chick as well as the rat (Table VII).

Table VII

Relative Provitamin A Activities of All-trans- -carotene and Pro- -carotene

Substance	Relative Potency	
	Chick	Rat
All-trans- β-carotene	100	100
All-trans- γ-carotene (prepared from pro- γ-carotene)	42	42
All-trans- γ-carotene (from tomato paste)	--	47
Pro- γ-carotene (from Pyracantha)	51	41

As shown by Table VII, the values for all-*trans*- γ-carotene are considerably higher than those (28, 26%) reported previously (7, this thesis), but the potencies in the chick and rat are identical. The values for pro- γ-carotene *versus* all-*trans*- γ-carotene (ex pro-γ-carotene) are the same in the rat but pro- γ-carotene has the higher potency in the chick. This is the first clear case reported where a *cis* isomer has equivalent or higher provitamin A activity than the all-*trans* form.

II. PHYTOFLUENOL, A NEW, NATURALLY OCCURRING POLYENE

A. INTRODUCTION

The resolution of extracts from a wide variety of plant materials has demonstrated the presence of a colorless hydrocarbon termed phytofluene (67,70,71,55,2), and its formula $C_{40}H_{64}$ ($\pm$ 2H), together with other experimental evidence (72), places it among the C_{40}-polyenes with a carotenoid-like structure. Phytofluene is colorless, containing seven double bonds of which only five are believed to be conjugated, but shows a strong, greenish-gray fluorescence in ultraviolet light.

Since phytofluene is more highly hydrogenated than the common C_{40}-carotenoids it may represent an intermediate stage in the biosynthesis of these pigments (55,2), since it occurs regularly in plant organs which are rich in carotenoids. The ratio of pigment to phytofluene, in the materials first studied, was always greater than unity. Some representative figures of the ratio of total carotenoids to phytofluene are: 100 : 1 in carrots; 15 : 1 in ripe tomatoes; and 10 :1 in the ripe berries of Pyracantha angustifolia Schneid.

An investigation of the blue, anthocyan-containing blossoms of the Brazilian tree, Jacaranda ovalifolia R. Br. (grown in California) revealed a so far unique case in which phytofluene was the major constituent of the polyene mixture. The total amounts extracted from 1 kg. of fresh blossoms were 0.6 mg. of phytofluene and 0.06 mg. of carotenoid pigments (almost exclusively β-carotene).

B. CHARACTERIZATION OF PHYTOFLUENOL

Since most of the common carotenoid hydrocarbons occur in nature along with varying amounts of their oxygenated derivatives, it would be expected that oxygen-containing, colorless polyenes might also be found. In working with commercial tomato paste, a new, colorless polyene was isolated whose properties indicate that it is an oxygenated phytofluene. Its amount is roughly 2% that of the phytofluene content and thus only a fraction of a per cent of the total polyenes present.

Although phytofluenol has not yet been isolated in analytically pure state, some of its properties indicate the presence of oxygen in its molecule. When partitioned between hexane and 83% aqueous ethanol, the ratio, concentration in the upper phase: concentration in the lower phase, was found to be 6 : 1 for phytofluenol, whereas the corresponding value for phytofluene is 100 : 0. In the partition between hexane and 95% methanol, the ratios were; 2.2 : 1 for phytofluenol, and 100 : 1 for phytofluene.

The adsorption behavior is also what one would expect for an oxygenated phytofluene (Table VIII). The difference between the adsorption affinities of phytofluene and phytofluenol is nearly the same as that between β-carotene, $C_{40}H_{56}$, and cryptoxanthin, $C_{40}H_{55}{\cdot}OH$. This indicates a difference of one hydroxyl group, as do the partition coefficients.

As the name suggests, phytofluenol has the same qualitative spectral characteristics as phytofluene (Figure 15). The wave length positions of the maxima and minima as well as their relative heights agree very well with those of phytofluene. The fluorescence of phytofluenol cannot be differentiated from that of phytofluene by the inspection of solutions or

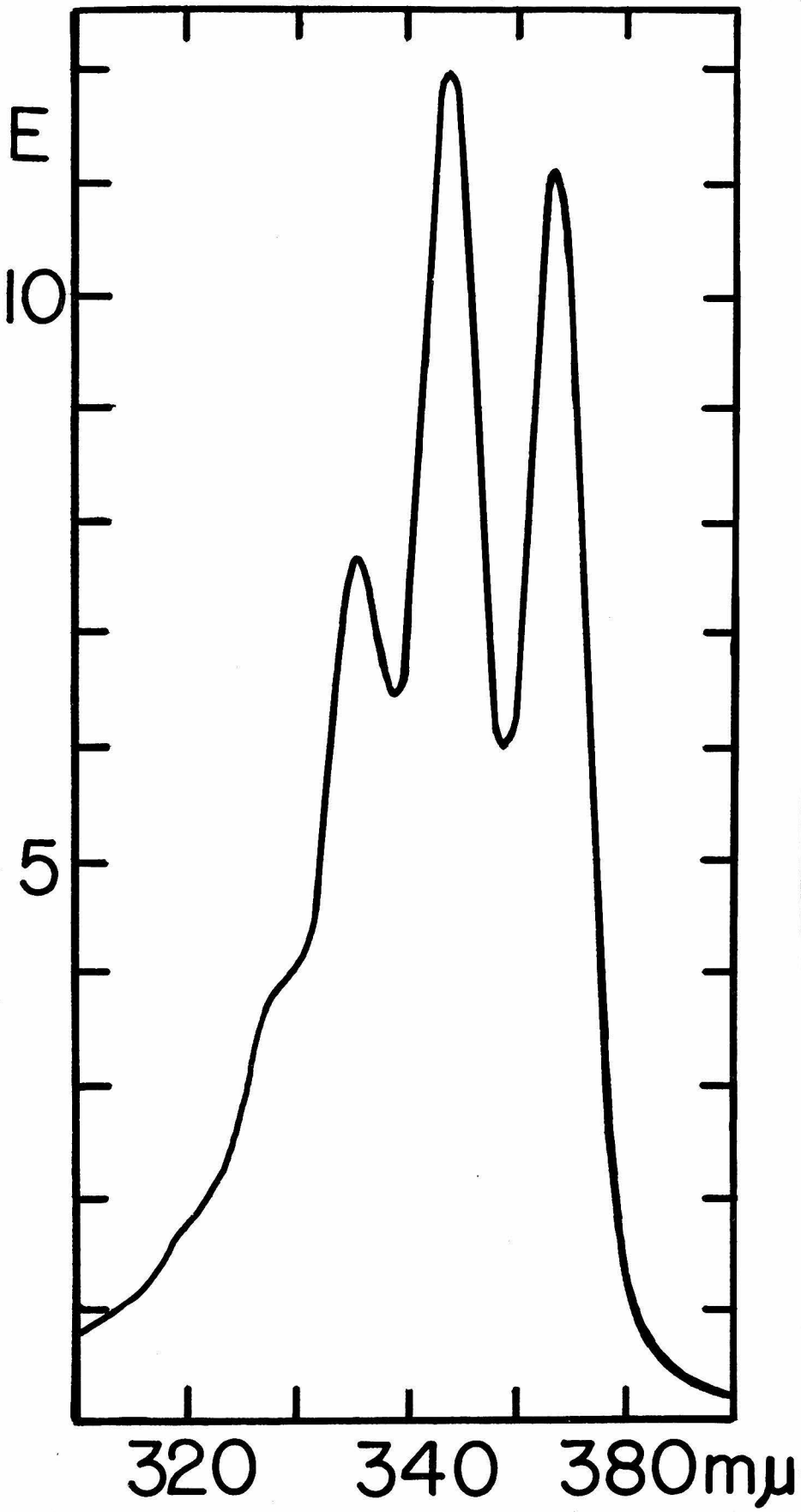

Figure 15. Extinction curve of phytofluenol in hexane as taken in the Beckman photoelectric spectrophotometer (E is relative extinction).

chromatographic columns.

Table VIII

Adsorption Sequence of Some Carotenoids and Phytofluenol on Lime

Developed with 15% acetone in petroleum ether	Developed with 1 to 3% acetone in petroleum ether
Lycopene	γ-Carotene
Neolycopene A	β-Carotene
Phytofluenol	α-Carotene
Cryptoxanthin	Phytofluene
γ-Carotene	Neo- α-Carotenes

Phytofluenol gives a bluish-green coloration on acid earths (filtrol) which is characteristic of phytofluene and many other polyenic compounds (13,36,72).

Phytofluenol could not be observed in extracts of "Tangerine" tomatoes or in a sample of fresh tomatoes (variety unknown). Since the commercial tomato paste used for this work is prepared from different amounts of two varieties of tomatoes ("Pearson" and "San Marzano"), it is possible that phytofluenol occurs only in one of the two varieties.

III. STEREOISOMERIC DIPHENYLBUTADIENES

A. INTRODUCTION

1. Stereoisomerism in Diphenylpolyenes

In contrast to the carotenoids, where many examples of stable cis-trans isomers are known, there is little evidence for the existence of stereoisomers in the higher members of the diphenylpolyenes $C_6H_5\cdot(CH{=}CH)_n\cdot C_6H_5$.* Kuhn (31) has expressed the opinion that this is due to a lack of suitable preparative methods. Ebel (12) believes that a rapid rate or rearrangement would explain why many predicted stereoisomers are not observed. This latter statement is in accordance with observations made by Zechmeister and LeRosen (57,58) in a study of diphenyloctatetraene, where, besides the stable all-trans forms, several labile steric forms were observed on the Tswett column. Methods for the synthesis of higher diphenylpolyenes, largely developed by Kuhn and Winterstein (32), gave only one steric form, even when the synthesis was carried out by several different methods. The synthetic products were shown to have the all-trans configuration in the cases of diphenylhexatriene and diphenyloctatetraene, by means of X-ray diffraction studies carried out by Hengstenberg and Kuhn (19,20).

Kuhn and Winterstein (32) developed mathematical formulas for calculating the number of possible stereoisomers, N, of the symmetrical, unbranched diphenylpolyenes containing n aliphatic double bonds.

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* In all further discussion the name diphenylpolyene is understood to refer to the class of compounds with this general formula.

When n is even $N = 2^{n-1} + 2^{\frac{n}{2} - 1}$ and for

n odd $N = 2^{n-1} + 2^{\frac{n-1}{2}}$

Expressed in slightly different form, these are the formulas used in calculating the number of sterically unhindered isomers in the case of carotenoids.

Although the above formulas are derived on the assumption that all of the aliphatic double bonds can undergo trans ⟶ cis shifts, Zechmeister and LeRosen (57,58) pointed out that such shifts would not be equally probable for all the double bonds. In particular, if the double bonds adjacent to the benzene rings were in the cis configuration, the hydrogen atoms of the benzene rings in the ortho positions to the side chain would be spatially hindered by certain hydrogen atoms of the aliphatic conjugated system. The same authors predicted, on this basis, that when an all-trans-diphenylpolyene stereoisomerizes, those isomers would be favored which have a trans configuration at both terminal double bonds of the aliphatic system.

2. Cis and trans Stilbenes

Two spatial forms of stilbene were first observed in 1897, when Otto and Stoffel (42) obtained the cis-isomer. Cis-stilbene ("isostilbene") has been prepared chemically by reduction of diphenylacetylene, $C_6H_5 \cdot C \equiv C \cdot C_6H_5$, and by decarboxylation of α-phenylcinnamic acid, $C_6H_5 \cdot CH=C(C_6H_5) \cdot COOH$. A trans ⟶ cis rearrangement can also be brought about by irradiation with ultraviolet light. The light absorption curves of cis and trans-stilbenes have been studied by Smakula and Wassermann (46), and the differences between the extinction curves of these two stereo-

isomers have been interpreted by Lewis and Calvin (34). The latter authors assumed that, in cis-stilbene, the steric conflict between the two ortho hydrogen atoms of the benzene ring forced the molecule out of coplanarity and diminished the resonance. The energy difference between cis and trans-stilbenes (10 kcal. per mole), as well as the different light absorption of the two steric forms, can be reasonably explained by the diminished resonance of the cis form.

B. STERIC FORMS OF 1,4-DIPHENYLBUTADIENE

1. Earlier Investigations on Diphenylbutadiene

The two expected cis isomers of diphenylbutadiene were prepared by Straus (47) as early as 1905. The cis-cis isomer was obtained by reduction of diphenyldiacetylene, and the cis-trans form by a reduction of trans-1,4-diphenylbutenine, $C_6H_5 \cdot C \equiv C \cdot CH = CH \cdot C_6H_5$. trans-trans-diphenylbutadiene could be obtained from either of the other stereoisomers by irradiation or by some reduction process. Straus obtained the cis-cis form as crystals and the cis-trans isomer as an oil.

Kelber and Schwartz (26), also Ott and Schröter (41) were able to confirm the work of Straus using other preparative methods. Zechmeister and Sandoval (73) made a study of some spectral changes which occurred when solutions of trans-trans-diphenylbutadiene were irradiated with artificial light or "insolated" (exposure to sunlight). These authors were able to isolate, by chromatographic methods, a stereochemically homogeneous cis isomer. The quantitative extinction curves were determined for the trans-trans compound and for the cis isomer, as well as their behavior toward heat and iodine catalysis.

2. A Comparative Study of the Stereoisomeric 1,4-Diphenylbutadienes

Since the extension of the methods used in the field of the carotenoids should reasonably lead to a closer correlation between the synthetic and natural polyenes, the earlier synthesis of the three stereoisomeric diphenylbutadienes by Straus was repeated. However, modern methods of chromatography and spectrophotometry were used to isolate and differentiate the three possible stereoisomeric forms.

The synthesis of cis-cis-diphenylbutadiene was carried out by the

original Straus procedure but also by a partial catalytic hydrogenation of diphenyldiacetylene, $C_6H_5 \cdot C \equiv C \cdot C \equiv C \cdot C_6H_5$, using a palladium-barium sulfate catalyst.

The cis-trans isomer was prepared in the manner described by Straus (47), and the product purified by chromatography. The method of irradiation used by Zechmeister and Sandoval (73) was also applied for the preparation of the cis-trans form, and it seems to be preferable to the reduction method used by Straus.

Spectral Characteristics of the Three Stereoisomers - The profound influence of the stereochemical configuration on the spectral properties of a polyene is illustrated by the extinction curves of the three steric forms (Figures 16, 17).

The very large differences in the character of light absorption for the diphenylbutadienes are probably due to the fact that both the cis-trans and the cis-cis isomers are of the spatially hindered type. While in the case of lycopene, a single trans $\longrightarrow$ cis rotation shifts the position of the main maximum 7.5 mμ toward shorter wave lengths, (in hexane, measured in the Beckman spectrophotometer), one trans $\longrightarrow$ cis shift in the diphenylbutadienes causes a wave length decrease of the main maximum of 14-15 mμ (Table IX), which is in good accordance with values reported for stilbene, where the corresponding decrease in the wave length position (in ethanol) is 15 mμ (46). The data given in Table IX constitutes additional evidence which supports the assignment of configurations by Straus (47). The lack of fine structure in the fundamental band of the two cis isomers can be correlated with similar observations made in the study of poly-cis-lycopenes (page 28).

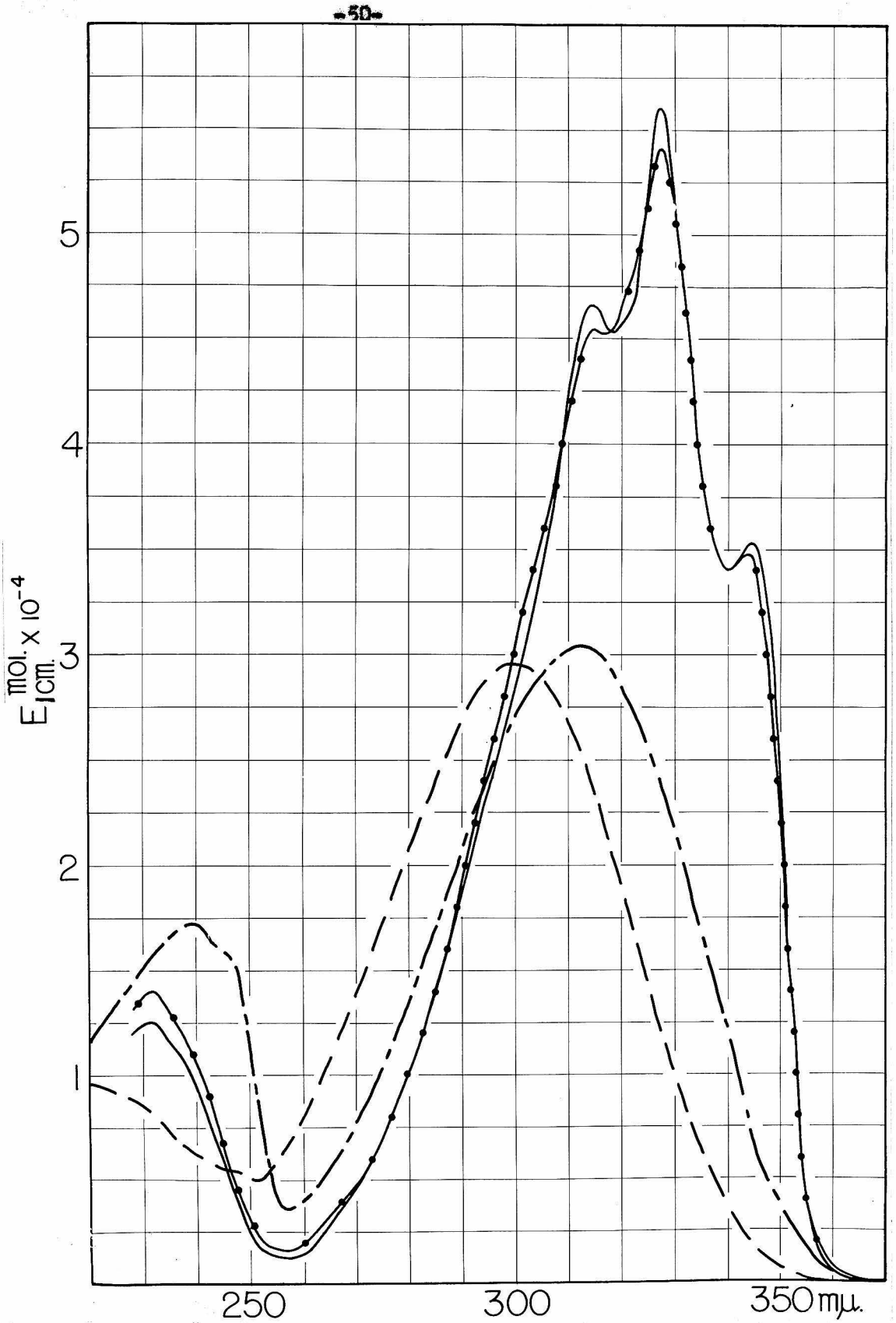

Figure 16. Molecular extinction curves of the three stereoisomeric diphenylbutadienes in hexane: ——, trans-trans compound; —— - ——, cis-trans compound; ----, cis-cis compound; ——·——·——, after iodine catalysis of any of the foregoing solutions.

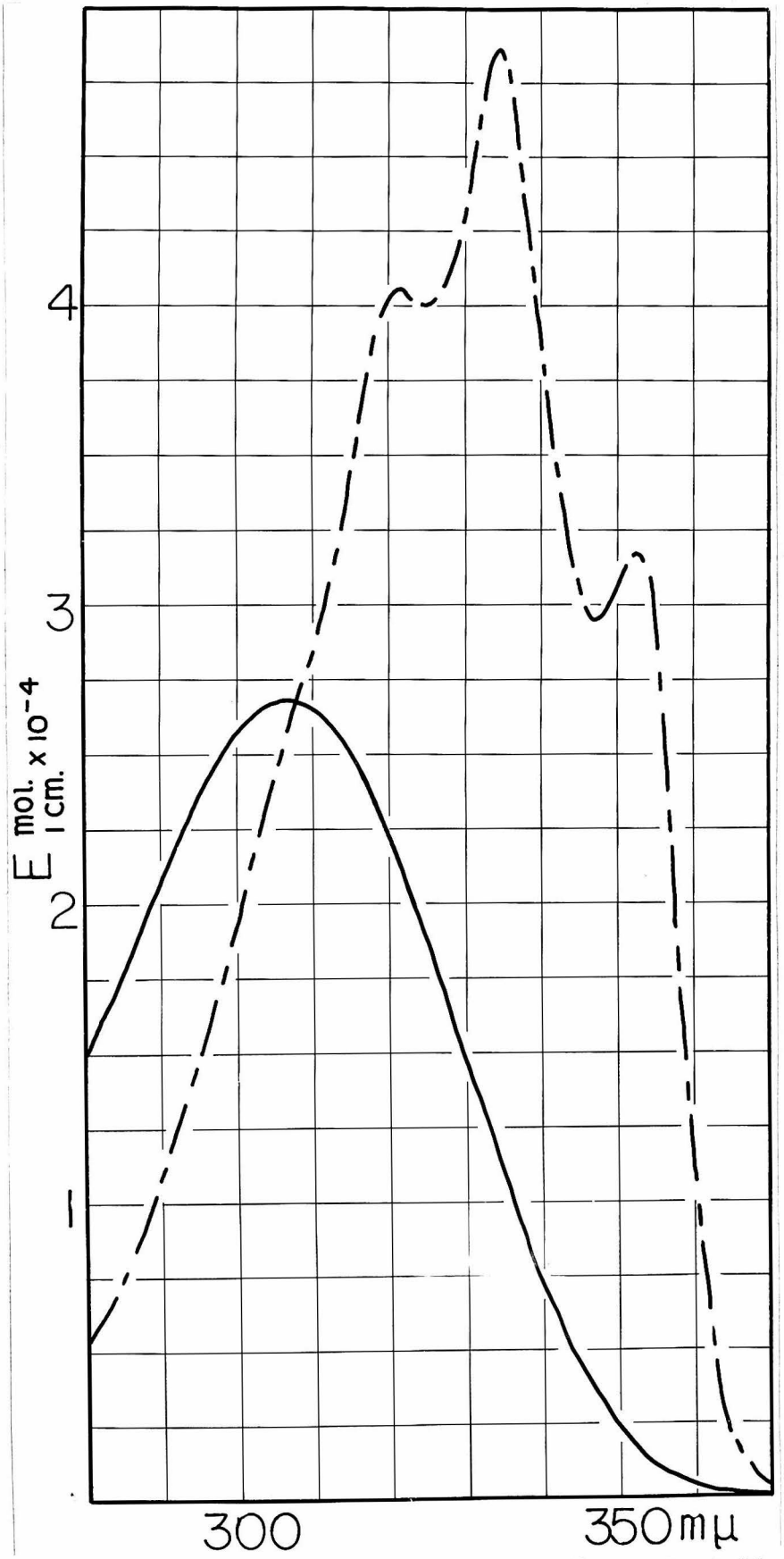

Figure 17. Molecular extinction curve of cis-cis-diphenylbutadiene in benzene; ——, fresh solution; —— - —— -, after iodine catalysis.

The models of the three diphenylbutadienes given in Figure 18 show that the overall shape of the cis-trans molecule is quite different from that of the trans-trans or the cis-cis form. The opened V-shape of the mono-cis isomer should give rise to a characteristic absorption band in the spectrum, as is observed in the case of a carotenoid stereoisomer with a similar configuration. Although a distinct cis-peak does not appear in the spectral curve of cis-trans-diphenylbutadiene it has the highest extinction of the three steric forms in the region between 230 and 240 mμ, (cf. Figure 16). This wave length region is approximately where a cis-peak would be expected. In the case of the C_{40} carotenoids, the wave length difference between the positions of the longest wave length maximum and the cis-peak is about 142 mμ (in hexane) but the corresponding figure for cis-trans-ciphenylbutadiene is only about 105 mμ. In view of the fact that the resonating system is much shorter in the latter case, the value of 105 mμ is reasonable.

The decrease in the intensity of the fundamental band, as measured by the area beneath the absorption curve, closely parallels the decrease in intensity which one finds in poly-cis-lycopenes (page 27).

Chromatographic Sequence - The chromatographic sequence of the three stereoisomeric diphenylbutadienes observed on an alumina column, using petroleum ether as a developer, is, from top to bottom; trans-trans, cis-trans, and cis-cis. This decrease in adsorption affinities with increasing number of cis double bonds is characteric for the lycopene set and for most cis-isomers in the other carotenoid hydrocarbon sets.

Fluorescence Characteristics - The differences in the fluorescence of the three steric forms of diphenylbutadiene are of great help in the

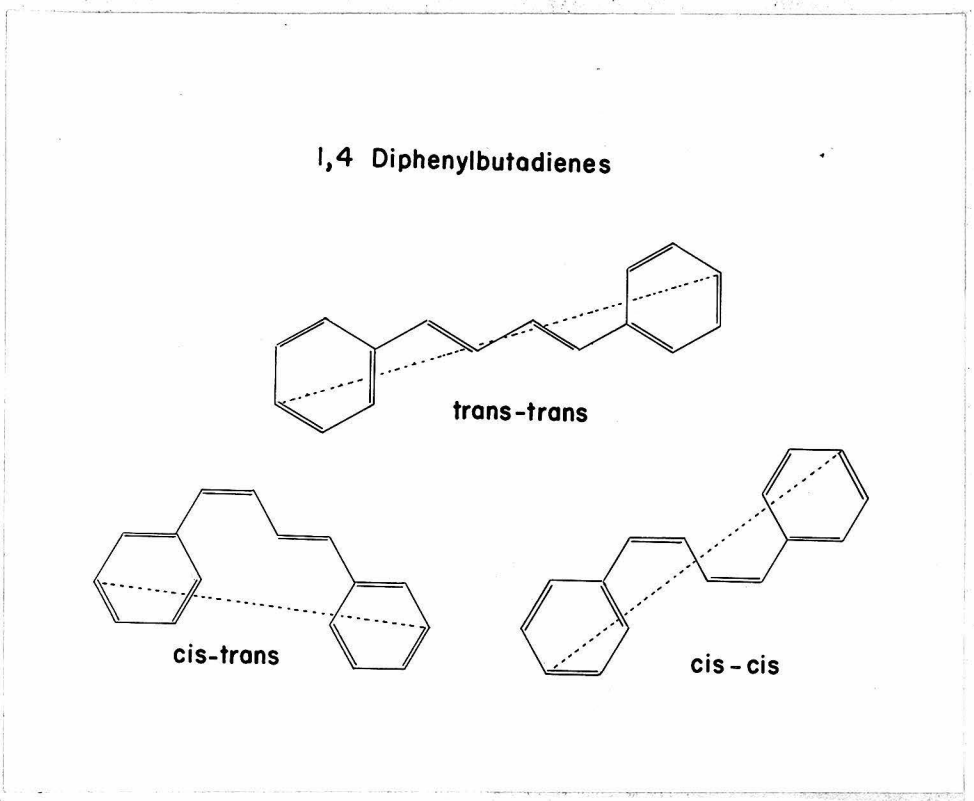

Figure 18. Models of trans-trans- (top), cis-trans- (bottom, left), and cis-cis-diphenylbutadiene (bottom, right). (Values: C=C, 1.33 Å; C-C, 1.46 A.; and C=C-C angle 124^0 $20'$. The dotted lines connect the ends of each resonating system).

location of zones on the chromatogram. The zone of the trans-trans form is easily identified by its intense bluish fluorescence in ultraviolet light. The cis-trans and cis-cis forms do not fluoresce either on the Tswett column, or in solution, but on alumina their zones can be located easily, since they quench the weak fluorescence of commercial alumina. Zones of all three stereoisomers on the chromatographic column also may be located by brushing the extruded column with permanganate solution (61).

The liquid chromatogram method (using alumina and petroleum ether) proved to be useful for a sharp separation of the cis-trans and cis-cis isomers. The filtrate from the column was collected in suitable fractions and a few drops of each fraction were tested by adding a drop of dilute iodine solution (in petroleum ether) and illuminating for a few minutes. If either of the cis forms is present, an inspection with ultraviolet light will then reveal fluorescence caused by the catalytic formation of trans-trans-diphenylbutadiene. This test is sensitive enough to permit the detection of one to two micrograms in a two-ml. sample.

Heat Stability - The stability of the three forms to heat was tested in the following ways. In petroleum ether solution, all three compounds are stable for at least two months when stored at 4°. When hexane solutions (b.p. 67°) are refluxed in an all-glass apparatus in darkness for forty-five minutes, all three forms are unchanged. In another set of experiments, samples of each steric form were sealed in evacuated pyrex tubes and kept at 205° for ten minutes in darkness. The trans-trans and cis-trans compounds were practically unaffected and the cis-cis form showed less than 5% isomerization.

Photo-Stability - The behavior of the three stereoisomers toward light

was tested in two different types of experiments: a) illumination of hexane solutions (20 mg. per liter) in pyrex flasks with a 250 watt light bulb for several hours, and b) exposure of more dilute hexane solutions (3-4 mg. per liter) to intense sunshine, in transparent quartz tubes, for one to ten minutes. In the a) series the cis-trans-isomer remained practically unchanged, even after seventeen hours of illumination, at which time, the cis-cis and trans-trans isomers (Figures 19, 20) had been almost quantitatively converted to the cis-trans isomer. The conversion of the trans-trans isomer was nearly complete after two hours illumination, while the cis-cis form required thirteen to fourteen hours under the same conditions. No significant photochemical destruction of any of the three isomers occurred during a seventeen hour period of illumination.

In series b) the trans-trans isomer was largely converted to the cis-trans form by a one minute insolation, and almost completely after ten minutes exposure (Figure 21). The cis-cis isomer was almost completely converted to the cis-trans configuration after ten minutes isolation (Figure 22), while the cis-trans form was practically unchanged under these conditions. There was 8-10% photo-destruction in these experiments.

In both series the extent of irreversible changes was estimated by carrying out an iodine catalysis after the completion of the experiment, and calculating the final concentration based on known equilibrium values of the stereoisomeric mixture obtained by catalysis of any of the three steric forms (Table XII). This mixture consists of about 97% trans-trans and 3% cis-trans form, while no cis-cis form has been observed so far under these conditions.

The necessity for light in effecting iodine catalysis of carotenoids

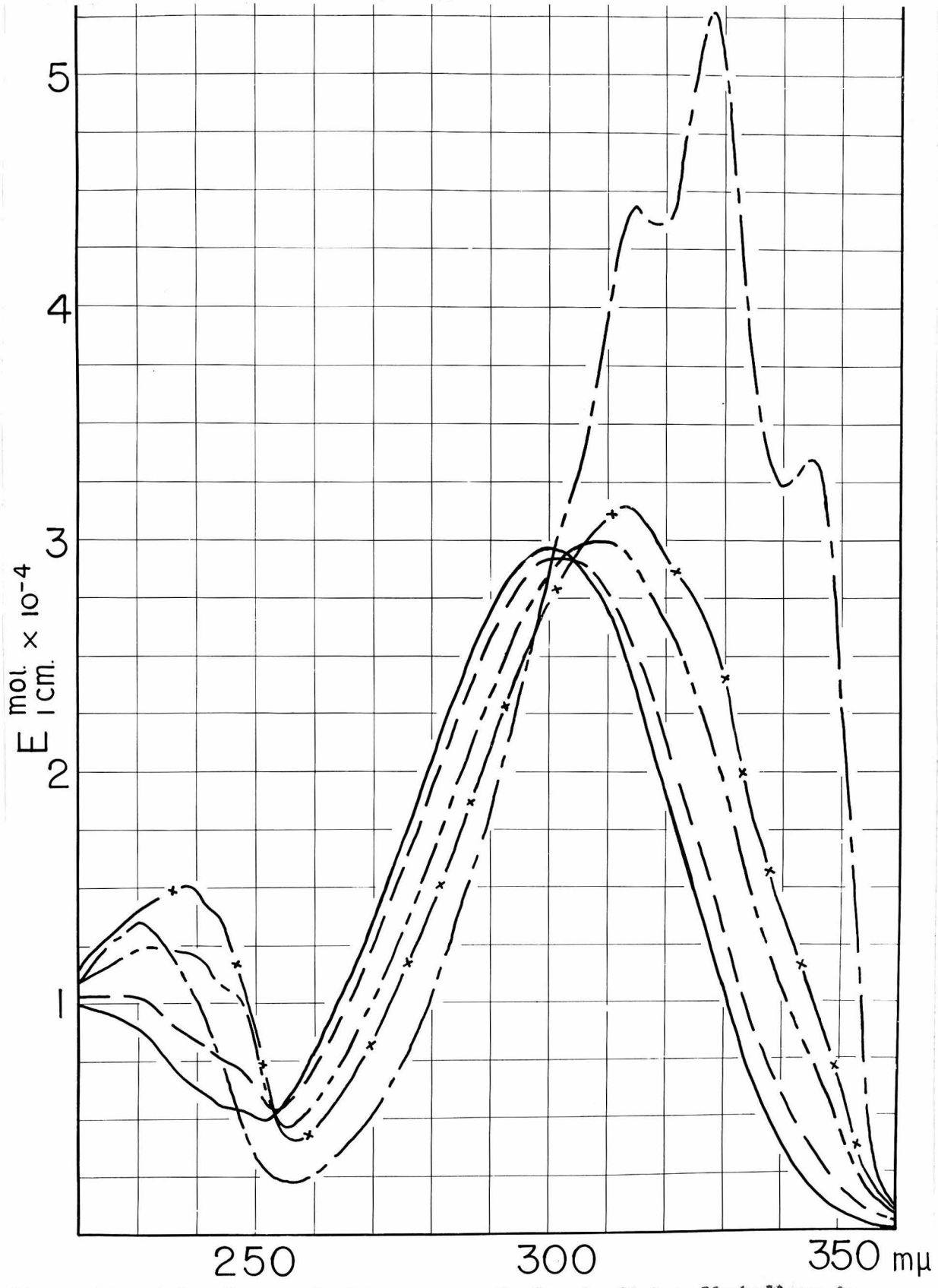

Figure 19. Molecular extinction curve of cis-cis-diphenylbutadiene in hexane, and its shift toward the curve of the cis-trans form during illumination with a 250-watt bulb: —, fresh solution — — —, after forty-five minutes; — — — —, three hours and forty-five minutes; and —x—x—, seventeen hours of illumination — — — —, after iodine catalysis at the end of seventeen hours of illumination.

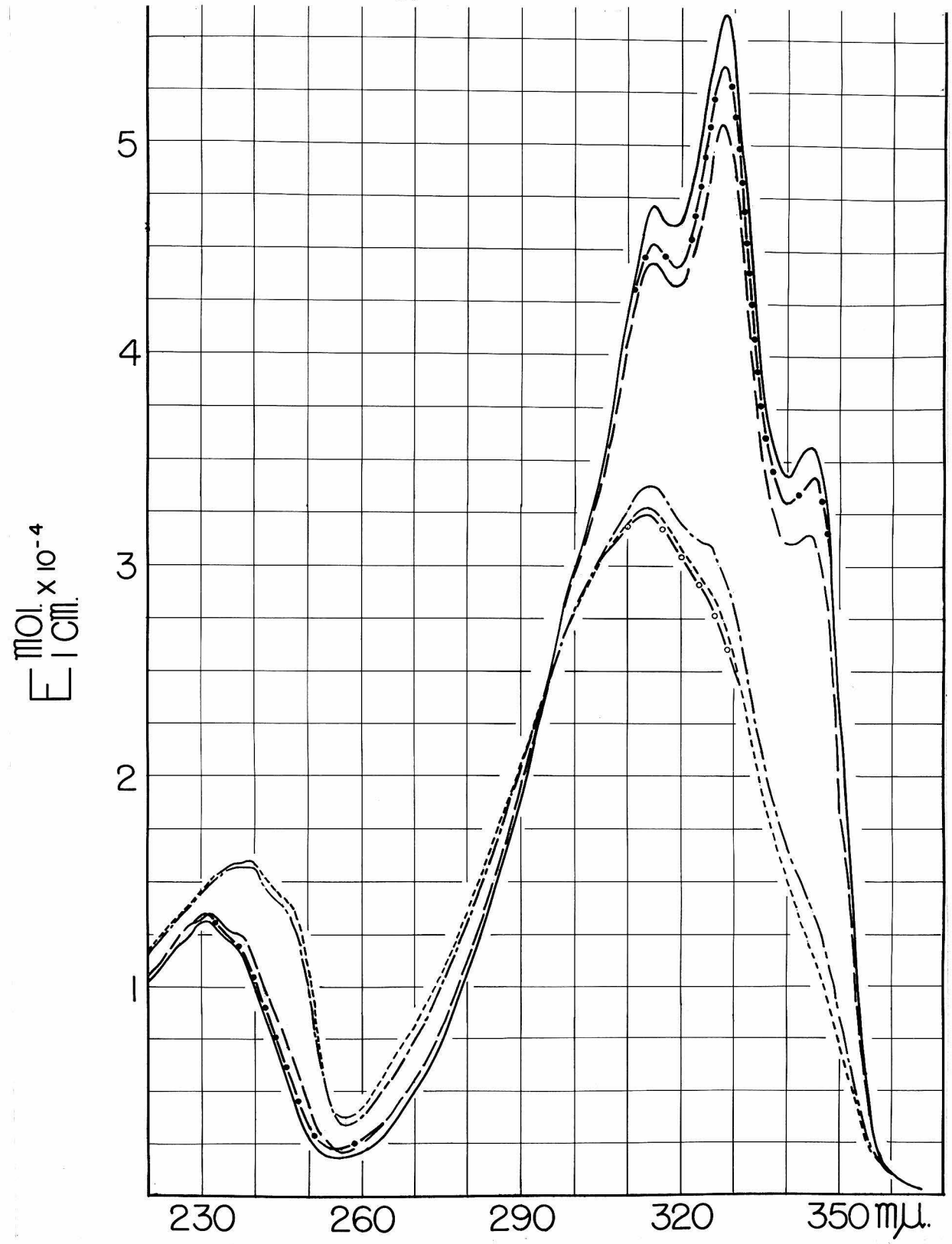

Figure 20. Molecular extinction curve of trans-trans-diphenylbutadiene in hexane, and its shift toward the curve of the cis-trans form during illumination with a 250-watt bulb: ——, fresh solution of the trans-trans compound; — — —, after fifteen minutes; —·—·—, forty-five minutes; ----, one hour and forty-five minutes —o—o—, eight hours illumination; —●—●—, after iodine catalysis at the end of seventeen hours illumination.

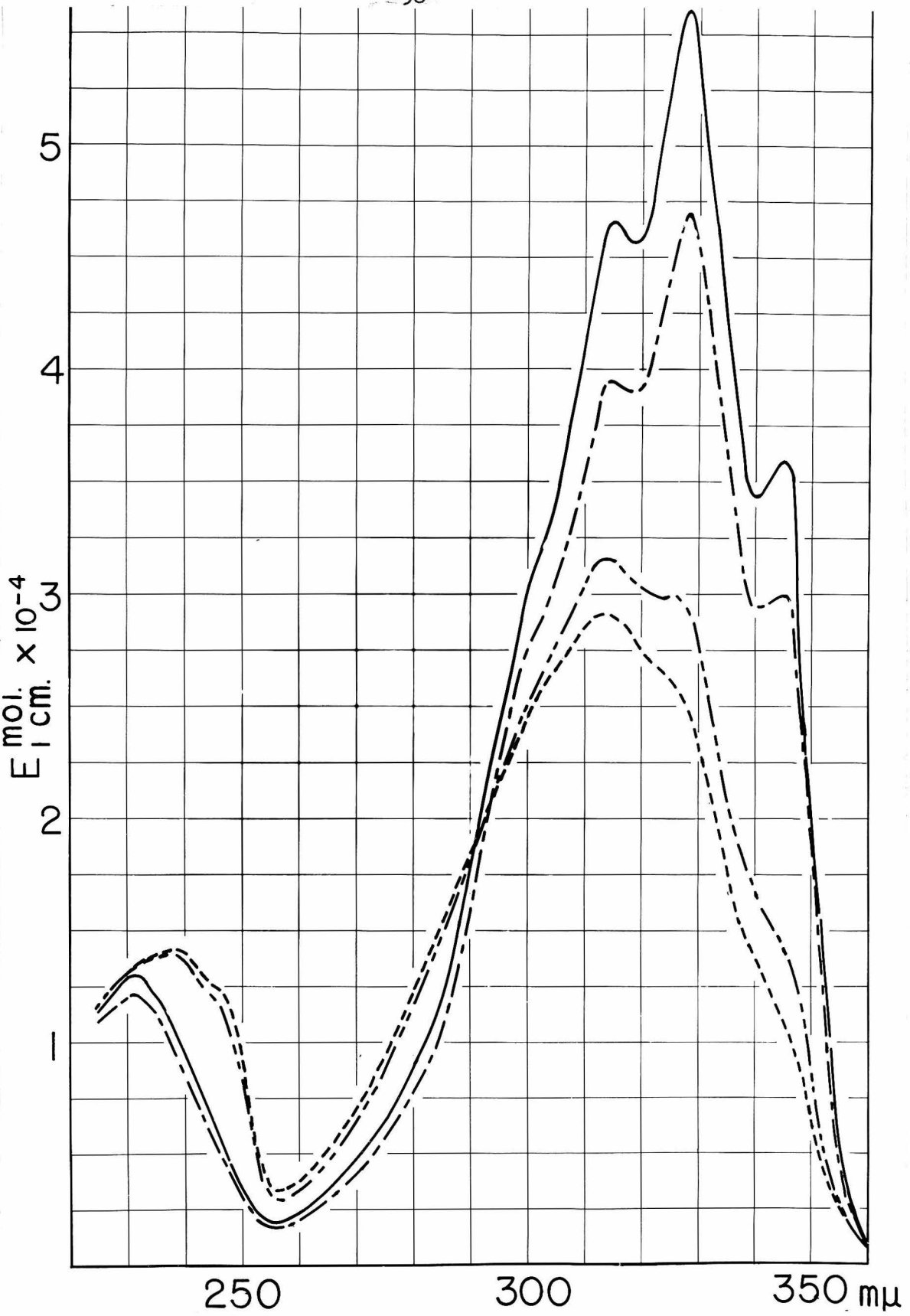

Figure 21. Molecular extinction curve of trans-trans-diphenylbutadiene in hexane, and its shift toward the curve of the cis-trans form during exposure to sunshine: —, fresh solution; — - - —, after one minute; ----, ten minutes of insolation; —·—·, after iodine catalysis at the end of ten minutes of insolation.

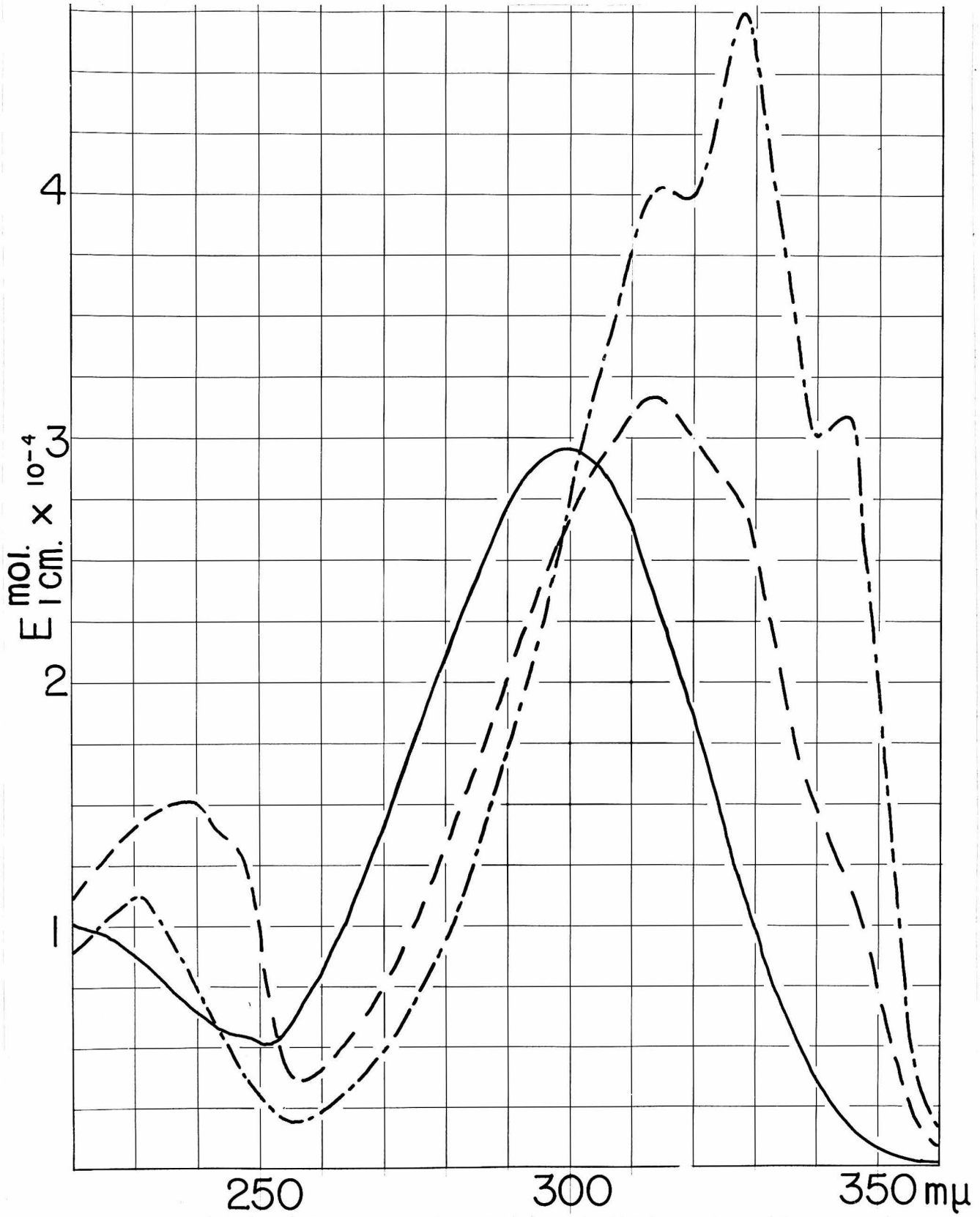

Figure 22. Molecular extinction curve of cis-cis-diphenylbutadiene in hexane, and its shift toward the curve of the cis-trans form during exposure to sunshine: ——, fresh solution; - - - -, after ten minutes of insolation; —·—·, after iodine catalysis at the end of ten minutes of insolation.

is well known (49). The same phenomenon takes place in the diphenylbutadiene set, as is illustrated in Figure 23. In darkness, after the addition of iodine to a hexane solution of cis-cis diphenylbutadiene, there was no observed change within half an hour. Exposure of this solution to light caused a rapid conversion. Some idea of the mechanism of this rearrangement was obtained by taking the complete extinction curves after definite times of illumination. Figure 23 shows that the process, cis-cis $\longrightarrow$ cis-trans $\longrightarrow$ trans-trans proceeds in a stepwise fashion. The appearance of any significant amount of the trans-trans isomer in the early stages of this process would be manifested by the appearance of bulges or peaks at those wave lengths where the maxima of the trans-trans form are located.

Degree of Stability of the Solvent-free Stereoisomers - Although cis-trans-diphenylbutadiene appears to be the most stable of the three steric forms when illuminated in dilute solution, it is the least stable in the solvent-free condition. The trans-trans and cis-cis forms crystallize easily, but attempts to crystallize the cis-trans compound failed and yielded an oil, which, on exposure to light, deposited crystals of the trans-trans isomer. The arrangement of molecules of cis-trans-diphenylbutadiene into a crystal grating might be much more difficult than for the other members of this set, considering their bent shape, and could account for the failure to obtain crystals.

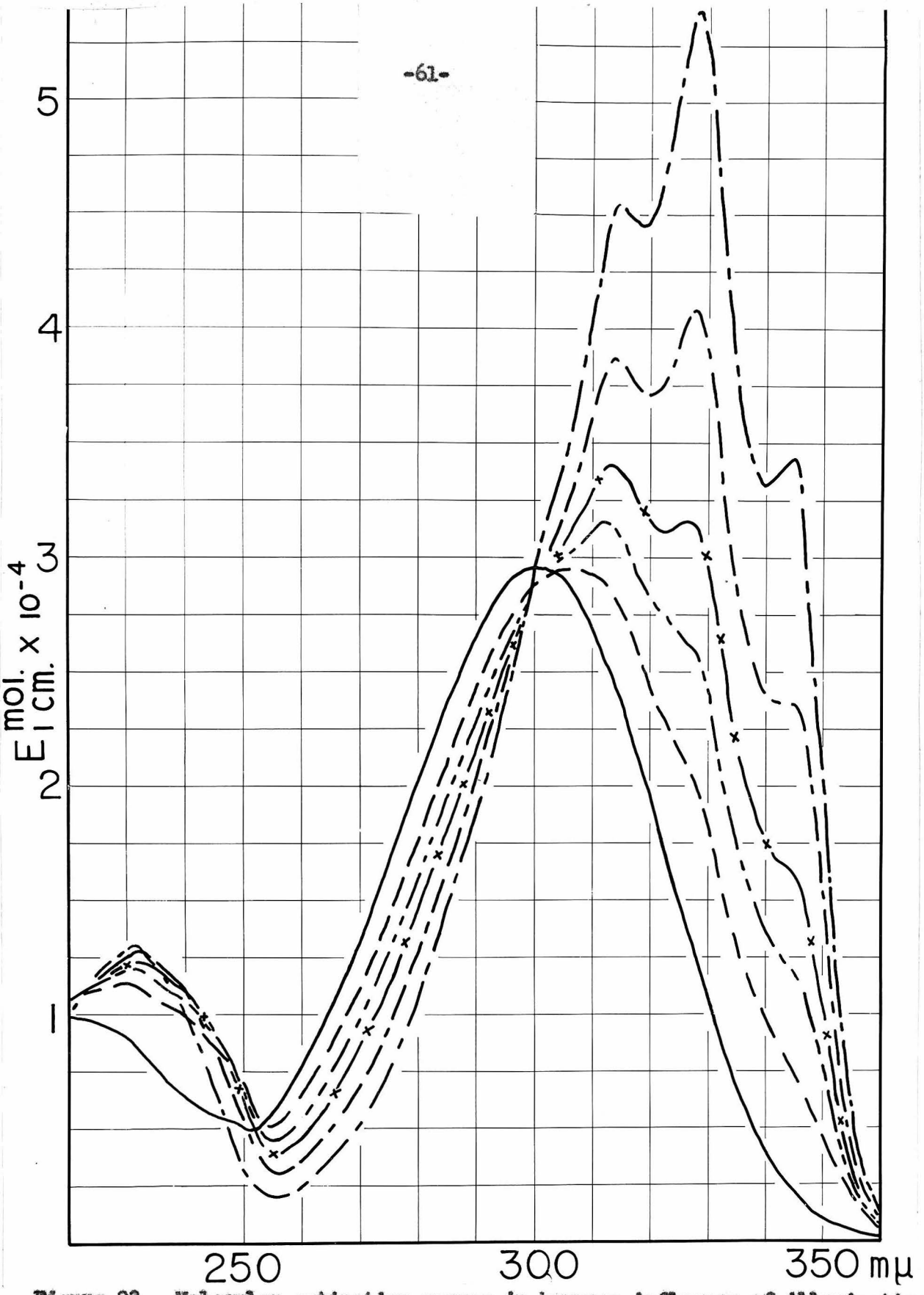

Figure 23. Molecular extinction curves in hexane; influence of illumination on the stereoisomerization of cis-cis-diphenylbutadiene caused by iodine catalysis: —, fresh solution with iodine, kept in darkness for thirty minutes; ----, after eighty seconds; — - - —, one hundred and forty seconds; —x—x—, 200 seconds; — — - — —, 320 seconds; and — - — -, 25 minutes illumination.

Acknowledgment - This work was carried out under the guidance of Professor L. Zechmeister and has been published in part, (5,18,62,63, 64,80). I wish to thank Mr. Börge Wille and Dr. A. Sandoval for their co-operation in many phases of the work. Professor H. J. Deuel Jr. and his group carried out the bio-assays for provitamin A activities. I am indebted to Professor F. W. Went and Professor E. G. Anderson for supplying experimental material, and to Professor A. J. Haagen-Smit, the late Dr. G. Oppenheimer and Mr. G. Swinehart for microanalyses. Mr. Kenneth Koe and Mr. Lawrence Wallcave gave technical assistance.

I. ISOLATION OF SOME *cis* CAROTENOIDS

A. INTRODUCTION

In all of the experimental work reported in this Thesis, certain standard techniques, equipment, and reagents were used. In order to avoid a useless repetition of detail, a description is now given which applies to all further work, unless specific mention is necessary for a particular case.

Adsorbents - Two brands of calcium hydroxide were used; Shell Brand, Chemical Hydrate, 98% through 325 mesh, Westvaco Chlorine Products, Newark, Calif. (no longer available) and Sierra Hydrated Lime, Superfine, United States Lime Products Corp., Los Angeles, Calif. It was sometimes necessary to mix the limes with Celite, #545, Johns-Manville Co., in order to obtain a better rate of filtration.

The alumina used was Activated Alumina, Grade F, 80-200 mesh, Aluminum Ore Co., East St. Louis, Ill. The alumina was re-ground to -200 mesh, and mixed with celite.

The "silicic acid" was Merck reagent silicic acid plus 20% celite.

Solvents - Petroleum ether was used in most chromatographic experiments. The brand was Skellysolve B, boiling range 60°-70°. Benzene used for chromatographic work was technical grade, and that used for spectra and crystallization was reagent grade. Acetone and ethanol refer to c.p. acetone and to 95% ethanol. A commercial grade of methanol was used. Hexane used for spectral work was prepared by treating Phillips commercial grade hexane, or Eastman practical grade hexane repeatedly with fuming sulfuric acid until its optical density was near that of

distilled water.

Chromatographic Columns. - Unless otherwise stated, the term "column" refers to a 30 x 8 cm. cylindrical column.

Spectral Measurements - Visual spectra were taken on an Evaluating Grating Spectroscope, Zeiss, light filter BG-7. Quantitative extinction curves were taken, using a Beckman photoelectric spectrophotometer (4). Measurements were customarily made by using average values based on two independent weighings.

General Notes - Evaporations and concentrations were performed in vacuo (water aspirators) while a stream of nitrogen or carbon dioxide bubbled through the solution. Sintered glass funnels were used for elutions, and in general, where possible, all-glass apparatus was used for evaporations, washing etc. Melting points were taken on an electrically heated Berl block in capillary tubes. The capillary tubes were either sealed under vacuum or filled with carbon dioxide and then sealed.

Iodine Catalysis - Iodine (1% of the pigment weight) was added to the solution in volumetric flasks and fifteen minutes of illumination followed, using two 3500° Mazda white fluorescent lights (40 watt, length of the tubes 120 cm.; from a 60 cm. distance).

B. CAROTENOIDS FROM PYRACANTHA

Extraction - Fresh Pyracantha berries, picked in Southern California, in December and January, were stored under methanol in darkness at 4° until they were worked up. It was found that, under these conditions, even half a year of storage would not essentially alter the results. Sixteen kilograms of this material (fresh weight) was drained of methanol and the moist berries were coarsely cut in an electric chopper. The material, after having been kept under fresh methanol for twenty-four hours, was centrifuged and the greenish-brown liquid discarded. The extraction was carried out with 1.5-kg. portions by shaking mechanically for fifteen minutes with 1 liter methanol + 1 liter petroleum ether, which formed two liquid phases. The residue was allowed to dry on trays for five-six hours and then ground in a Wiley mill No. 1 until the particles passed a 1.5 mm. sieve. The extraction was repeated three more times, as described, and all extracts were combined. The polyenes contained in the methanol layer were transferred by cautious addition of water (in 20-liter separatory funnels) into the petroleum ether (30 liters) which was then washed methanol-free. The dark red solution was dried over anhydrous calcium chloride and concentrated to 1.5 liters. When kept at 4° overnight, a greenish gummy material appeared which was removed by sucking the solution through a 4-cm. layer of calcium carbonate on a sintered glass funnel. The adsorbent was washed with a small volume of petroleum ether and the latter added to the main solution.

Isolation - The petroleum ether solution just mentioned was developed with the same solvent on twenty-one columns of Shell lime. Each column showed the following sequence after half an hour of development.

(Figures on the left denote width of zones in mm.; some very narrow, pale interzones are omitted.

2 brown

2 red

5 orange

17 red-orange, lycopene

40 pale orange, undifferentiated

17 red-orange

40 orange, prolycopene

18 yellow

6 pale interzone

36 orange, pro-γ-carotene

7 yellow

2 orange

65 orange, β-carotene

10 yellow

8 fluorescent; partly overlapping preceding zone

The development was continued until nine-tenths of the -carotene zone had been washed into the filtrate. (This filtrate contained considerable amounts of phytofluene). Each column then showed the following sequence

---

3 brown

6 orange — Section I

7 red-orange, lycopene

40	pale orange, undifferentiated	
23	red-orange	Section II
48	orange, prolycopene	
20	very pale yellow	
42	orange, pro-γ-carotene	Section III
5	yellow	
4	pale orange	
6	almost colorless	Section IV
10	orange, β-carotene	

After elution, the corresponding Sections I, II, III, and IV of the twenty-one columns were combined.

The above Section I consisted mainly of all-trans-lycopene which was estimated photometrically.

1. Pro-γ-carotene - The petroleum ether solution from Section III was rechromatographed on three columns. The combined main zones were eluted, washed free of alcohol and dried. The solution was then completely evaporated and the residue dissolved in a minimum amount of benzene. Pro-γ-carotene was obtained by adding absolute methanol dropwise at 20-25°, with stirring, until crystallization started, cooling in ice water and cautious addition of methanol up to three times the initial volume. After recrystallization at room temperature and washing the sample with 3 x 10 ml. of hot methanol in the centrifuge tube, 211 mg. of pure pro-γ-carotene was obtained, m.p. 134-5°. In a mixed chromatogram test there was no separation from an authentic sample.

2. Prolycopene - The eluate of Section II (from 21 columns), after

transfer into petroleum ether, was rechromatographed on twelve columns.

10	pale orange	
35	almost colorless	
2	yellow	
1	purple	
10	pale orange	
2	yellow	Section V
1	purple	
30	pale orange	Section VI
4	pale interzone	
8	red-orange	
1	pale interzone	
10	red-orange	Section VII
1	pale interzone	
6	red-orange	
35	orange, prolycopene	Section VIII
8	yellow	Section IX
3	orange	

Section VIII was rechromatographed on three columns. Upon development with 4% acetone in petroleum ether, the three main zones were combined and, after transfer into petroleum ether, the latter was evaporated to dryness. The residue was crystallized as given above for pro- γ -carotene; after crystallization, 735 mg. of pure prolycopene, m.p. 111.5 - 112.5°, was obtained, which did not separate, in a mixed chromatogram test, from an authentic sample.

Section VI was developed on three columns with petroleum ether + 4% acetone.

2	brown
8	red-orange
4	pale interzone
80	dark orange, poly-cis-lycopene I
100	several minor zones (added to Section VII)

3. Poly-cis-lycopenes I - III

The three combined 80 mm. zones were rechromatographed on a single column. The main zone of this chromatogram was transferred into petroleum ether and evaporated. The crystallization of its residue was carried out as for pro- γ-carotene (yield, 45 mg. of poly-cis-lycopene I; after recrystallization, m.p. 93-95°).

The total of Sections VII (from 12 columns) was developed on twelve columns with petroleum ether + 8% acetone.

8	pale orange	
6	purple	
4	pale interzone	
10	pale orange	
6	pale interzone	
4	pale orange	
10	pale interzone	
12	dark orange	
6	pale interzone	
56	pale orange	
18	red-orange	Section X
4	yellow	

3	pale interzone	
40	red orange	Section XI
8	pale yellow	
40	pale red	Section XII
16	pale orange	

Section X (from 12 columns) was developed with 6% acetone in petroleum ether on three columns and gave the following sequence.

15	several minor zones	
30	colorless	
80	pale orange	Section XIII
40	red-orange	Section XIV
30	several minor zones	

Section XIII (from 3 columns) was developed with 6% acetone in petroleum ether on a single column. The main zone was transferred into petroleum ether and evaporated. The residue was crystallized from benzene and methanol. For the crystal photograph in Figure 25, poly-cis-lycopene II was crystallized from petroleum ether + methanol with addition of enough ethanol to prevent two liquid phases. The yield after recrystallization was 14 mg. of poly-cis-lycopene II, m.p. 85-87°.

Section XIV (from 3 columns) was developed on a single column with petroleum ether and 6% acetone. The solution from the combined main zone was treated as just described. Yield, after recrystallization from benzene-methanol, was 19 mg. of poly-cis-lycopene III, m.p. 105-106°.

4. All-trans- γ-carotene

Section XI (from 12 columns) was developed on four columns with

petroleum ether and 4% acetone. The combined main zones were eluted, transferred into petroleum ether, evaporated and crystallized. After recrystallization, 42 mg. of all-trans- γ-carotene was obtained with the constant m.p. 128-129°. In the mixed chromatogram test it did not separate from an authentic sample.

5. Neo- γ-carotene P

Section XII (from 12 columns) was developed on three columns with petroleum ether and 6% acetone. The three combined main zones were transferred into petroleum ether. After a like development on a single column, the solution from the main zone was evaporated and the residue was crystallized. After recrystallization, 47 mg. of neo- γ-carotene P, m.p. 89-90°, resulted. The main pigment obtained by iodine catalysis from this isomer did not separate from γ-carotene (ex Mimulus) in the mixed chromatogram test.

6. Poly-cis-lycopenes IV - VI

Section IX was developed on three columns with petroleum ether and 2% acetone.

5	brown
2	colorless
18	several minor zones
100	orange, prolycopene
60	yellow (lower part orange)
8	red-orange, pro- γ-carotene
10	yellow

After transfer into petroleum ether, the three combined 60-mm. zones were developed with petroleum ether and 4% acetone on two columns.

15 several minor zones

130 bright yellow

40 yellow and pale orange zones

4 red-orange

The two combined 40-mm. zones were developed on a single column (25 x 4.5 cm.) with petroleum ether and 4% acetone.

10 several minor zones

6 colorless

60 pale yellow, poly-cis-lycopene IV

5 almost colorless

80 yellow, poly-cis-lycopene V

3 pale yellow

20 yellow-orange, poly-cis-lycopene VI

Each poly-cis compound was rechromatographed. Since the quantity of these pigments was not sufficient for the preparation of crystalline samples, in order to determine the extinction curves, the solvent was displaced from the columns with 65 ml. of optically pure hexane. After elution, the respective pigments were transferred into pure hexane. The concentrations were determined, after iodine catalysis, using the equilibrium value $E_{1\ cm.}^{mol.} = 14.6 \times 10^4$ at 468-469 mμ which is the average of the figures obtained with four different isomers and only slightly different from some data given earlier (59).

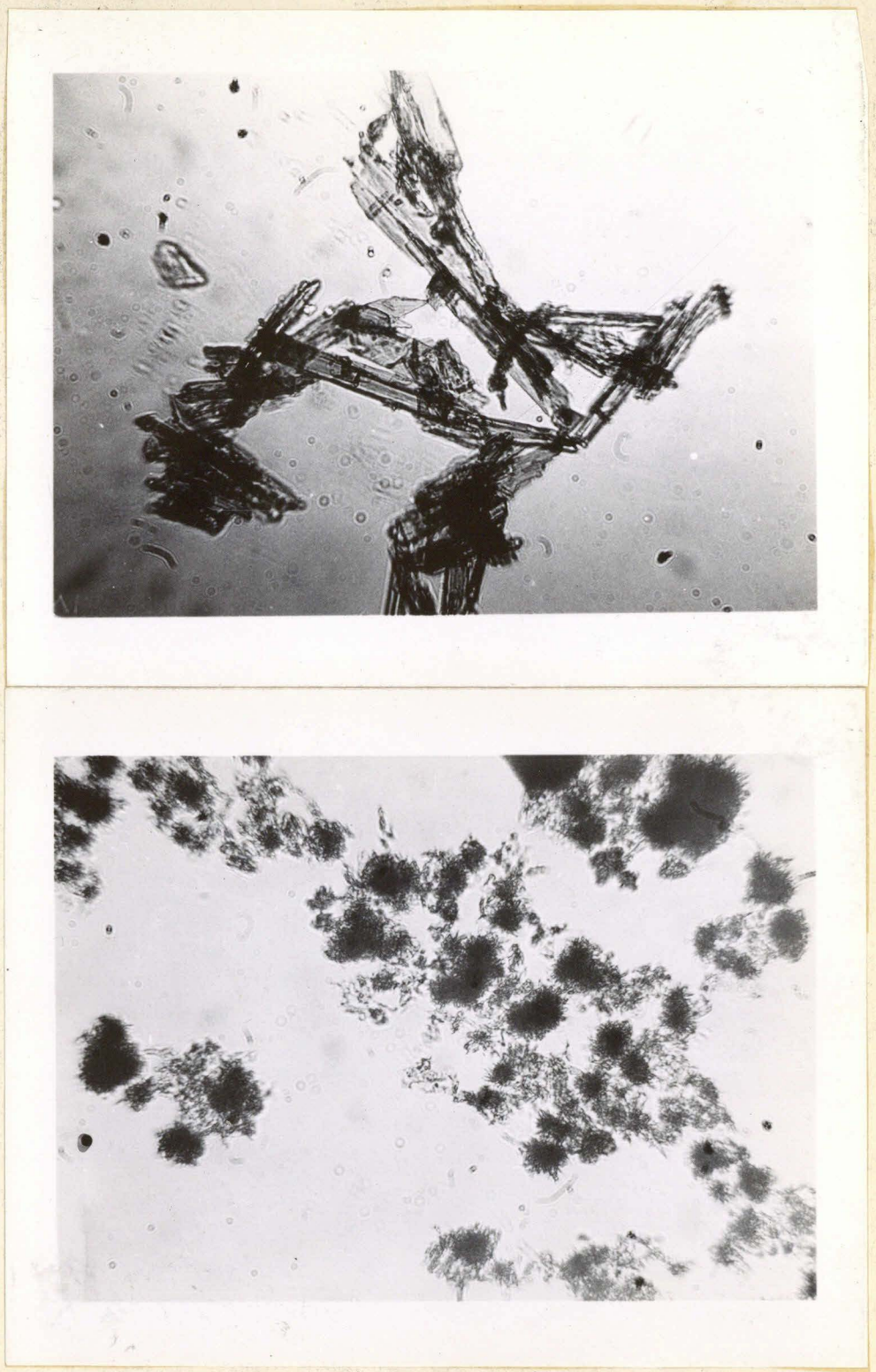

Figure 24. Prolycopene (upper) and Poly-cis-lycopene I (lower), crystallized from benzene and methanol. Magnification 430X.

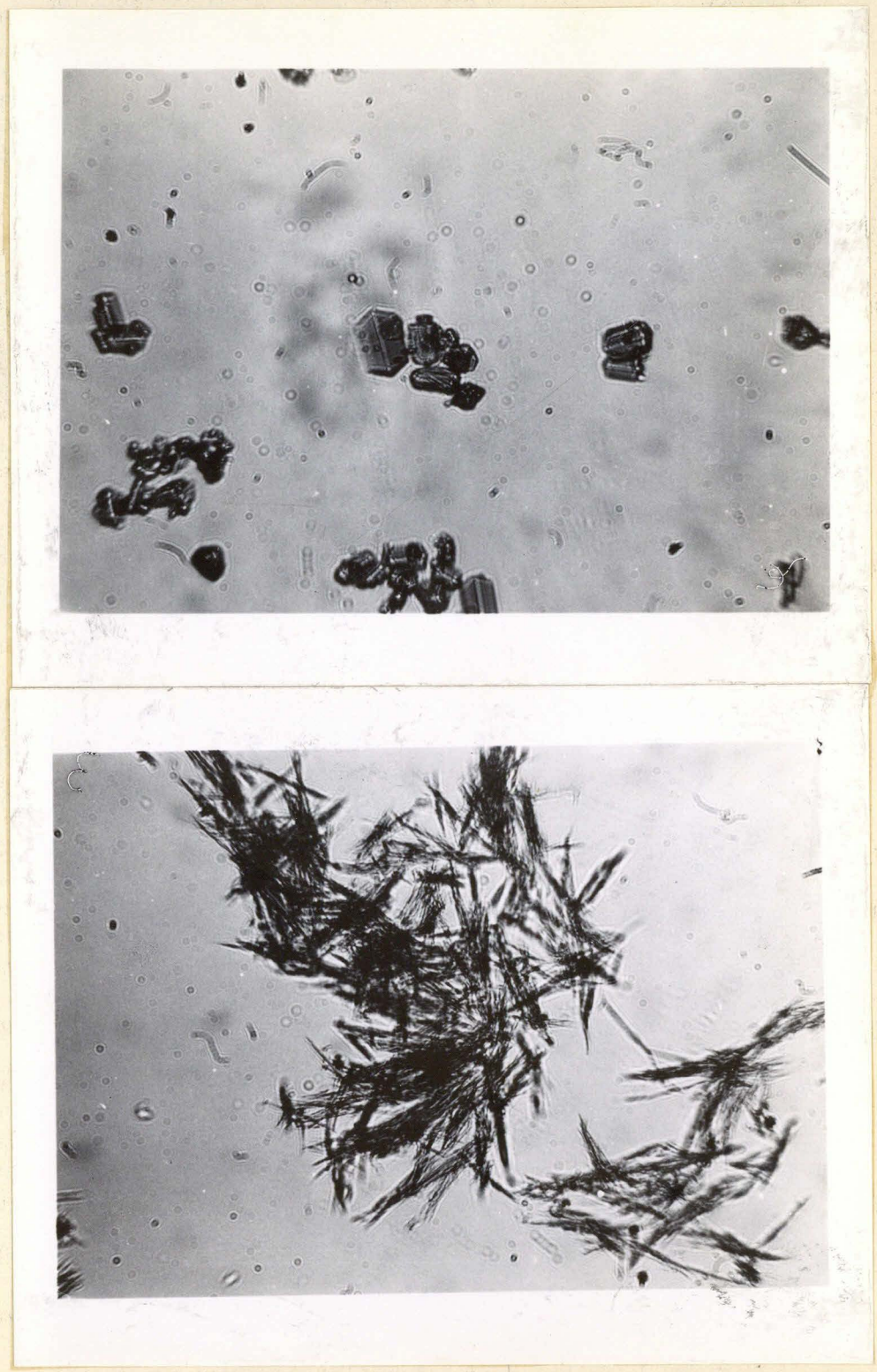

Figure 25. Poly-cis-lycopene II (upper), crystallized from petroleum ether and methanol, Poly-cis-lycopene III (lower), crystallized from benzene and methanol. Magnification 430X.

Table X

Molecular Extinction Coefficients of Some Poly-*cis*-lycopenes (*ex* *Pyracantha*) and their Stereoisomeric Equilibria at the Maxima (*italicized*) and Minima, in Hexane.

Fresh solution		After iodine isomerization	
mμ	mol. E x 10^{-4} 1 cm.	mμ	mol. E x 10^{-4} 1 cm.
		Poly-*cis*-lycopene I	
		498	12.0
		485	9.3
472	12.3	*467-8*	14.2
458-9	10.5	449	9.9
444-5	12.3	*442*	10.1
		375	1.4(4)
		361	2.8(6)
		351	2.0(3)
		347	2.0(7)
311	0.6(4)	317	0.7(2)
296	1.4(4)	*295*	3.6(0)
290	1.2)1)	265	1.2(0)
255	2.7(8)	*255-6*	1.4(5)
240	1.9(2)	241	1.0(9)
233	2.0(3)	*226*	1.6(6)

Table X

-Continued-

Molecular Extinction Coefficients of Some Poly-*cis*-lycopenes (*ex* *Pyracantha*) and their Stereoisomeric Equilibria at the Maxima (*italicized*) and Minima, in Hexane.

Fresh solution		After iodine isomerization	
mμ	mol. E x 10^{-4} 1 cm.	mμ	mol. E x 10^{-4} 1 cm.
		Poly-*cis*-lycopene II	
		498	12.0
		485	9.3
465-6	10.6	*467*	14.2
453-4	9.9	449	10.0
441-2	11.1	*442*	10.2
		375	1.5(5)
		361	3.0(2)
		351	2.1(5)
		346	2.2(1)
312	0.8(1)	316	0.7(6)
296	1.5(1)	*295*	3.6(9)
288-90	1.3(2)	265	1.2(1)
256	2.4(4)	*256*	1.4(2)
244	2.1(8)	242	1.0(2)
233	2.6(7)	*225*	1.5(1)

Table X

-Continued-

Molecular Extinction Coefficients of Some Poly-cis-lycopenes (ex Pyracantha) and their Stereoisomeric Equilibria at the Maxima (italicized) and Minima, in Hexane.

Fresh solution		After iodine isomerization	
mμ	mol. E x 10^{-4} 1 cm.	mμ	mol. E x 10^{-4} 1 cm.
		Poly-cis-lycopene III	
		498	12.4
		485	9.7
468	10.5	*467-8*	14.7
458-61	10.2	448	10.4
443-6	11.3	*442*	10.6
		375	1.6(7)
		361	3.1(0)
		351	2.2(4)
		347	2.2(8)
312	0.7(9)	316	0.8(2)
296	1.4(2)	*296*	3.8(2)
280-2	1.2(0)	266	1.2(6)
255-6	2.5(2)	*256-8*	1.4(8)
240	2.0(7)	242	1.1(0)
232	2.5(9)	*227*	1.7(1)

Table X

-Continued-

Molecular Extinction Coefficients of Some Poly-cis-lycopenes (ex Pyracantha) and their Stereoisomeric Equilibria at the Maxima (italicized) and Minima, in Hexane.

Fresh solution		After iodine isomerization	
mμ	mol. E x 10^{-4} 1 cm.	mμ	mol. E x 10^{-4} 1 cm.
		Poly-cis-lycopene IV	
		497	11.4
		485	10.0
		467	14.6
		449	11.8
426	10.6	442	11.6
412	8.9(7)	375	2.4
406	9.0(7)	*361*	3.4
		350	2.4(6)
		348	2.5(0)
315	1.2(7)	318	1.1
		295	4.2

Table X

-Continued-

Molecular Extinction Coefficients of Some Poly-cis-lycopenes (ex Pyracantha) and their Stereoisomeric Equilibria at the Maxima (italicized) and Minima, in Hexane.

Fresh solution		After iodine isomerization	
mμ	mol. E x 10^{-4} 1 cm.	mμ	mol. E x 10^{-4} 1 cm.
		Poly-cis-lycopene V	
		498	12.1
		485	9.6
		467-8	14.6
		449	10.4
431-2	8.9(8)	*442*	10.7
416-8	8.0(7)	375	1.8
412-4	8.1(3)	*361*	3.2(1)
		351	2.3(3)
		346	2.3(7)
314	1.1(8)	317	0.9(8)
		295	4.1

Table X

-Continued-

Molecular Extinction Coefficients of Some Poly-cis-lycopenes (ex-Pyracantha) and their Stereoisomeric Equilibria at the Maxima (italicized) and Minima, in Hexane.

Fresh solution		After iodine isomerization	
mμ	mol. E x 10^{-4} 1 cm.	mμ	mol. E x 10^{-4} 1 cm.
		Poly-cis lycopene VI	
		497-8	11.9
		485-6	9.8
		467-8	14.6
		448	10.7
433	8.1(6)	*442-3*	10.8
		374	1.7(6)
		361	3.0(7)
		350	2.2(8)
		346	2.3(3)
312-4	1.1(6)	316-8	0.9(5)
		296	3.8(4)

C. POLY-*Cis*-LYCOPENES FROM THE "TANGERINE" TOMATO

Extraction - 87.6 kg. of ripe tangerine tomatoes were sliced into quarters and ground with methanol in a "Waring Blendor" until a fine mash was obtained. This mash was filtered through a cloth lining in a basket centrifuge. The first liquid was found to be essentially free of carotenoid pigments and was discarded. The moist residue of tomato pulp was extracted by shaking 5 kg.-portions with 6 liters of 1 : 1 mixture of petroleum ether and methanol (there were two liquid phases) on a mechanical shaker for 20 minutes. The mixture was filtered through a cloth-lined basket in the centrifuge and the filtrate saved. The residue was extracted once more with a mixture of petroleum ether and methanol (2 : 1) and filtered as above. The residue (which was nearly colorless) was discarded. The combined filtrates were poured into 20-liter separatory funnels and the pigments were transferred to the petroleum ether by cautious addition of water. The aqueous phase was discarded. The petroleum ether solution was concentrated in vacuo to a total volume of about 3 liters. This concentrate was filtered through a 6 cm. layer of celite in a sintered glass funnel to remove gummy material. The celite was washed with 500 ml. of petroleum ether and the washings combined with the first filtrate.

Isolation - The petroleum ether solution of the pigments was chromatographed on fifteen columns and developed with 5% acetone in petroleum ether. The top to bottom sequence of the pigments on the column, after the lowest pigment zone was near the bottom, is given below. (Figures on the left denote width of zones in mm.).

15	pale buff	
20	2 red zones	Section I
5	pale orange	
4	very pale orange	
10	orange	
10	pale orange	Section II
22	orange	
10	yellow	
3	orange-red	
4	almost colorless	
52	orange (prolycopene)	Section III
20	yellow-orange	
30	pale yellow	
2	orange	
8	yellow-orange	Section IV
3	colorless	
8	orange (β-carotene)	
20	fluorescent zone (phytofluene)	
filtrate	fluorescent (phytofluene)	

Each column was divided into the sections indicated above and the corresponding eluates were combined.

The petroleum ether solution from Section I was chromatographed on two columns and developed with 10% acetone in petroleum ether solution. The two main zones were identified in the visual spectroscope as all-trans-lycopene and neolycopene A.

The petroleum ether solution of Section II was chromatographed on eight columns and developed with 6% acetone in petroleum ether. After development the following sequence was observed.

4	brown	
12	pale red	
113	dull orange	Section V
54	yellow-orange	Section VI
20	bright yellow	
4	red-orange	
10	orange	
30	colorless	

The sections indicated were cut and the eluates combined. A test in the visual spectroscope with iodine showed that only Sections V and VI contained poly-*cis*-lycopenes.

Poly-cis-lycopene I - The petroleum ether solution from Section V was rechromatographed on one column and developed with 6% acetone in petroleum ether. The solution of the main zone was evaporated to dryness in vacuo and the residue was dissolved in a minimum amount of warm benzene and transferred to a centrifuge tube. The pigment was crystallized by adding absolute methanol dropwise and cooling until crystallization started. A total of four volumes of methanol was added. After centrifuging and washing twice with methanol, the crystals were dried and recrystallized as above. Washing the crystals with hot methanol and decantation of the mother liquor was necessary to get rid of colorless impurities. The

third crystallization gave a material which was analytically pure. Anal. Calcd. for $C_{40}H_{56}$: C, 89.48; H, 10.52. Found: C, 89.63; H, 10.57. Yield of pure crystals was 9 mg.

A solution of the crystals in hexane showed the same maxima and minima as poly-cis-lycopene I which was isolated earlier from Pyracantha. A portion of the material was catalyzed with iodine and the main pigment zone was separated on the Tswett column. This zone did not separate from an authentic sample of lycopene in a mixed chromatogram test.

Poly-cis-lycopene III - The petroleum ether solution from Section VI was chromatographed on one column (alumina-celite 4: 1) and developed with 75% benzene in ligroin. The solution from the main zone was evaporated to small volume, diluted with petroleum ether and developed on one column with 6% acetone in petroleum ether. The solution from the main zone was evaporated to dryness in vacuo. The residue was dissolved in the minimum amount of warm benzene and transferred to a centrifuge tube. Crystallization was carried out by adding absolute methanol dropwise with cooling. After three crystallizations the yield was 17 mg. Anal. Calcd. for $C_{40}H_{56}$: C, 89.48; H, 10.52. Found: C, 89.37; H, 10.64.

The absorption spectrum was identical with poly-cis-lycopene III which was isolated earlier from Pyracantha. A portion of the crystals was catalyzed with iodine and chromatographed. The main pigment zone was separated and a mixed chromatogram test made with authentic all-trans-lycopene. There was no separation.

Prolycopene - The petroleum ether solution of Section III was chromatographed on six columns and developed with 3% acetone in petroleum ether. Each column showed the following sequence of zones.

3	brown	
125	pale buff	
75	orange	Section VII
30	bright yellow orange	
8	orange	
8	yellow	
35	colorless	

A test in the visual spectroscope showed that Section VII was the only zone containing a poly-cis-lycopene. The solution from Section VII was evaporated to dryness, dissolved in warm benzene and crystallized by adding absolute methanol slowly with cooling. After washing twice, recrystallizing and washing twice the yield of homogeneous crystals was 585 mg. The absorption spectrum identified the pigment as prolycopene.

A trial chromatogram of the solution from Section IV showed that it did not contain any appreciable amount of poly-cis-lycopene isomers and accordingly it was not worked up.

D. PREPARATION OF THE MATERIAL FOR BIOASSAYS

The isolation of all-trans- γ -carotene, neo- γ -carotene P and pro- γ -carotene was described on Pages 67 to 71 of this Thesis. A few milligrams of the analyzed crystals were dissolved in peroxide-free absolute ether, evaporated in a stream of CO_2 and dissolved in Wesson oil for the extinction curves and for the animal tests. Mixed neo- γ-carotenes. 12 mg. of γ-carotene in 50 ml. of petroleum ether was refluxed in an all-glass apparatus, in diffuse daylight for $\frac{1}{2}$ hour while nitrogen bubbled through. The solution was then developed on a 22 x 4.4 cm. calcium hydroxide column (Arrowhead Lime Products, mixed with celite 3 : 1) with petroleum ether containing 2% acetone. Below the orange-red main zone of unchanged all-trans form, a heterogeneous, much lighter zone appeared which was eluted with methanol. This eluate was kept at 0°C. while the upper zone was re-isomerized and chromatographed. After 4 such isomerizations, the combined mixed neo- γ -carotenes were transferred with water from methanol into petroleum ether and freed by chromatography from a small amount of all-trans contaminant. The main zone was then eluted with peroxide-free absolute ether and diluted to 150 ml. From this, 120 ml. was evaporated and dissolved in Wesson oil while three 10 ml. samples were necessary to estimate the concentration, as follows. Each of two such aliquots was evaporated, dissolved in 40 ml. of petroleum ether, refluxed for 30 min. as above, diluted to 100 ml. and estimated in the Beckman spectrophotometer on the basis of earlier data. The third 10 ml. aliquot, after evaporation, was dissolved in Wesson oil and extinction curves were taken (Figs. 26 and 27; see also Table XI).

During the bioassay period the oil solutions were kept in dry ice, in darkness, and showed practically no change in the extinction values. Details of the feeding experiments can be found in reference (8).

Table XI

Molecular Extinction Coefficients of Some Stereoisomeric -Carotenes in Wesson Oil at the Wave Length of Maximum Extinction.

Compound	Wavelength (mμ)	$E^{mol.}_{1cm.} \times 10^{-4}$
All-trans- γ-carotene	473-4	13.1
Neo- γ-carotene P	471-2	11.2
Mixed neo- γ-carotenes	466-7	9.5
Pro- γ-carotene	468-9	10.8

All-trans- γ-carotene was prepared from pro- γ-carotene by the following procedure. 150 mg. of pro- γ-carotene was dissolved in 80 ml. of petroleum ether in a 100 ml. pyrex volumetric flask and 4.5 mg. of iodine dissolved in ligroin was added. The solution was mixed and then irradiated with a 400 watt, water cooled projection lamp bulb at a distance of 10 cm. for 5 mins. The flask was shaken several times during the irradiation to mix the contents. The solution (a small amount of precipitate appeared) was chromatographed on one column (Sierra Lime-celite 3 : 1) and developed with 6% acetone in petroleum ether. The main, upper, red zone of all-trans- γ-carotene wad cut and eluted. The lower pale zones of neo- γ-carotenes were cut, eluted, and their petroleum ether solution was

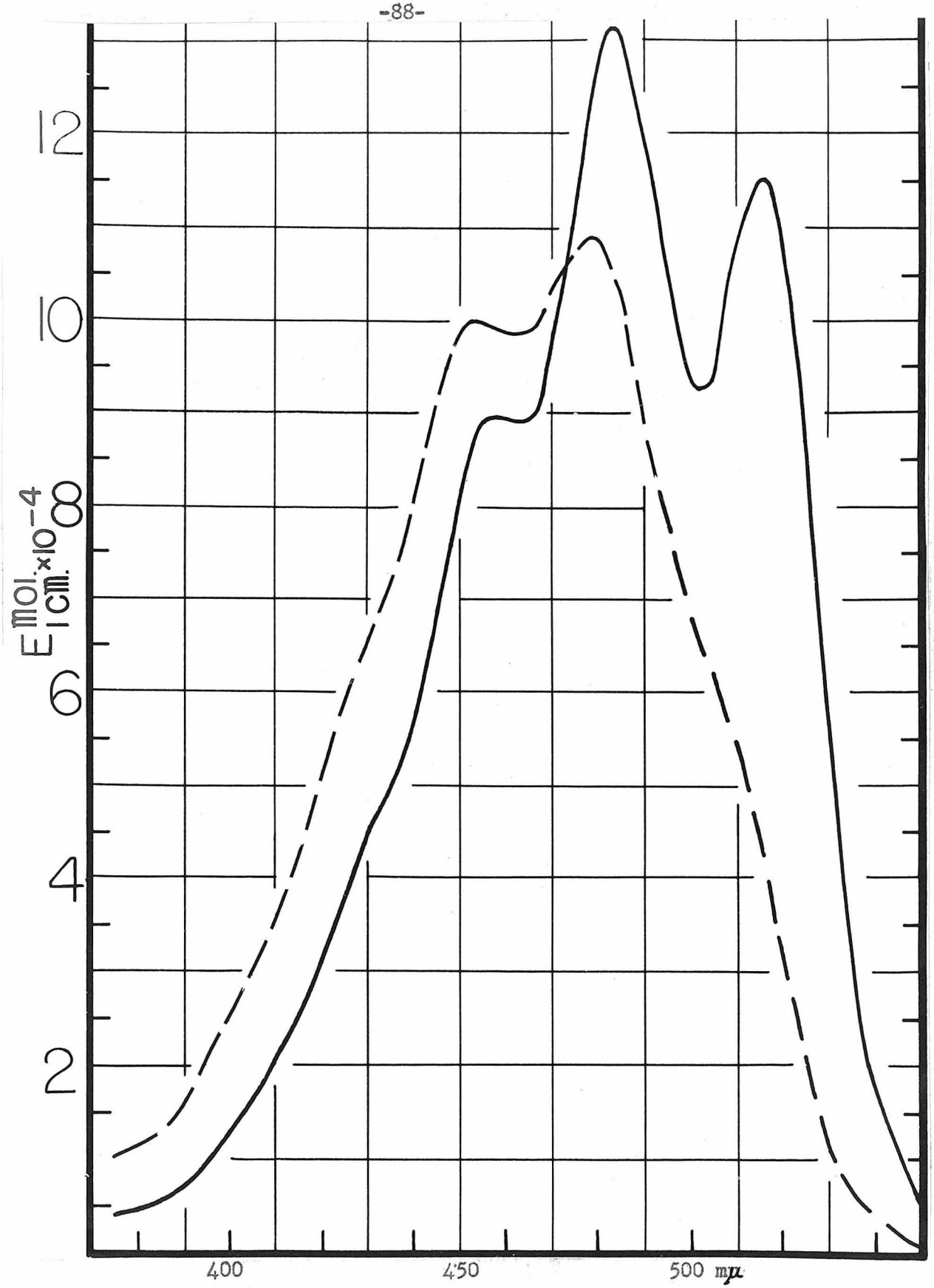

Figure 26. Molecular extinction curves of all-trans- γ -carotene (full line) and pro- γ -carotene (dashed line) in Wesson oil. Wavelengths in mμ.

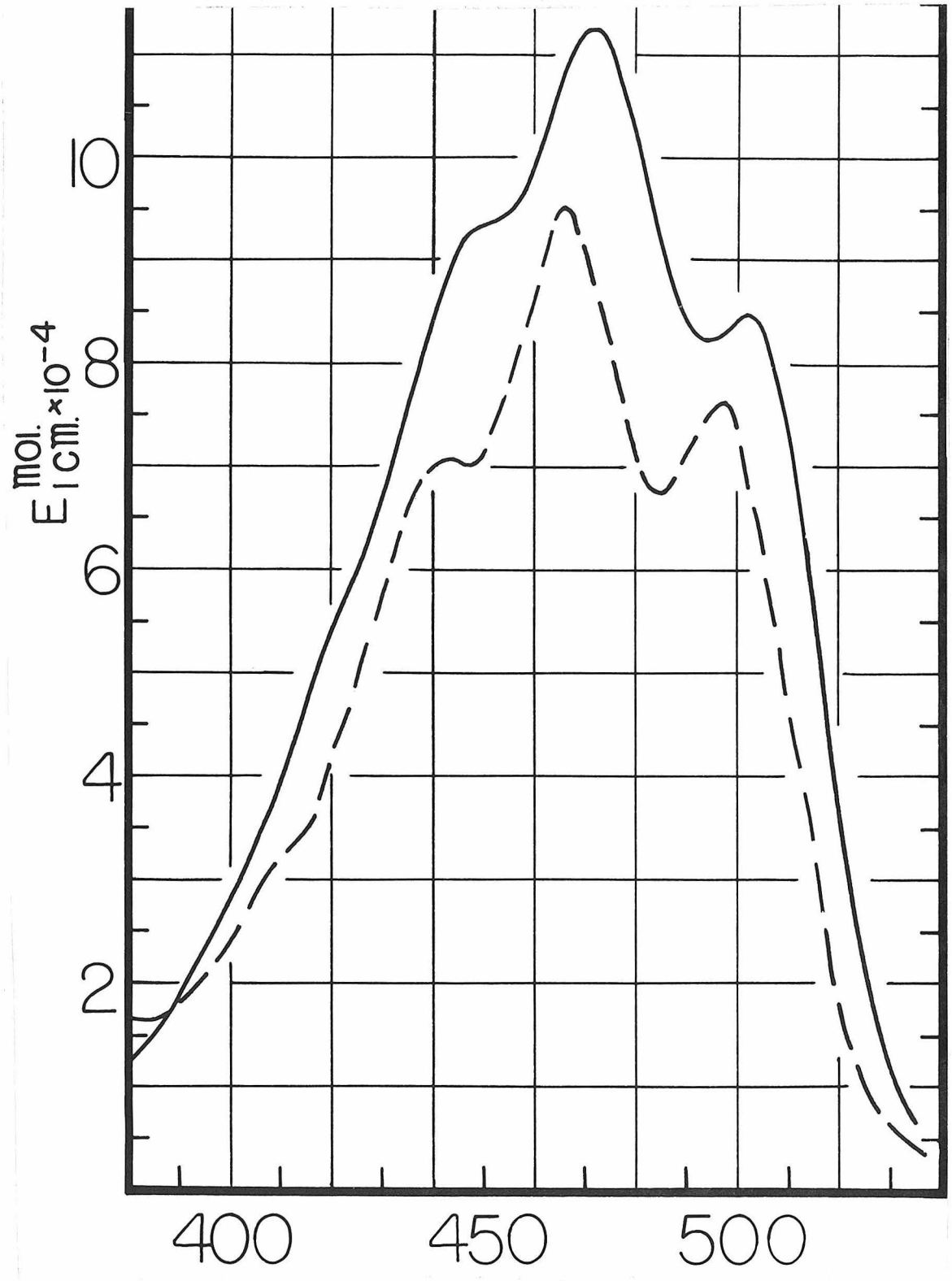

Figure 27. Molecular extinction curves in Wesson Oil of neo-γ-carotene P (full line) and of mixed neo-γ-carotenes (dashed line). Wavelengths in mμ.

treated again by the procedure just described. The combined eluates of all-trans- γ -carotene were transferred to petroleum ether, washed and dried. The petroleum ether solution was diluted to a composition of petroleum ether : benzene = 9 : 1 (total volume ca. 100 ml) and chromatographed on a 22 x 5 cm. column (alumina-celite 4 : 1). The column was developed with 60% benzene in petroleum ether. After development, the main zone was cut out, eluted, transferred to petroleum ether-benzene, washed and dried. The solution was filtered through a fine sintered glass funnel and evaporated to dryness in vacuo (water bath at 40°). The residue was transferred to a 15 ml. centrifuge tube with the minimum amount of pure benzene and crystallized by adding absolute methanol dropwise, with stirring, until crystals appeared. More methanol was added until ratio of benzene to methanol was 1 : 4. The centrifuge tube was cooled in ice-water mixture for three hours, centrifuged and mother liquor pipetted off. The crystals were washed once with benzene-methanol 1 : 4 and then with methanol. After drying, the crystals were redissolved in benzene and recrystallized twice by the above procedure. The final crystals were dried 2 hours in vacuum at the temperature of refluxing acetone. Molecular extinction values were calculated from two independent weighings. Found: $E^{mol.}_{1cm.}$ = 15.7 x 10^4 at 461 mμ in hexane and $E^{mol.}_{1cm.}$ = 14.2 x 10^4 at 473 mμ in Wesson oil. Anal. Calcd. for $C_{40}H_{56}$: C, 89.48; H, 10.52. Found: C, 89.79; H, 10.38. m.p. (cor.) = 136-137° Berl block. All trans- δ -carotene from tomato paste - This material had been saved from an earlier experiment. The chromatographic purification and crystallization followed the procedure just given. $E^{mol.}_{1cm.}$ = 15.9 x 10^4 at 461 mμ in hexane. m.p. 129-130° (cor.) Berl block.

In a mixed chromatogram test there was no separation of all-trans-carotene ex pro-γ-carotene and the γ-carotenes from tomato paste or commercial carotene.

The values for the Molecular Extinction coefficients of all-trans-γ-carotene in both Wesson oil and hexane are higher than previously reported (this thesis, 59) which would partially account for the higher activities found in the rat tests.

Pro-γ-carotene - The isolation was described on Page 67 of this thesis. The extinction coefficient in Wesson oil was: $E^{mol.}_{1cm.} = 10.8 \times 10^4$ at 468 mμ. This is identical with that reported earlier (Page 87 of this thesis).

II. COLORLESS POLYENES

A. ISOLATION OF PHYTOFLUENE FROM JACARANDA

920 g. of fresh blossoms of Jacaranda ovalifolia R. Br. were collected in June, 1948. The green stems were removed and the blossoms were covered with methanol. It was not necessary to grind the material. After standing overnight, the methanol (ca 3.5 liters) was drained off, mixed with a liter of petroleum ether (b.p. 60-70°), and water added cautiously to transfer the carotenoids to the petroleum ether phase. The aqueous phase was re-extracted with 500 ml. of petroleum ether and then discarded. The petroleum ether solutions were combined, washed, and dried. The filtered solution was concentrated in vacuo to a volume of 100 ml. This solution was chromatographed on a 25 x 4.5 cm column ($Ca(OH)_2$ - Shell Brand) and developed with ligroin. The following sequence was observed on the column from top to bottom (Figures on left denote width of zone in mm.):

3	fluorescent	
15	empty	
7	fluorescent	
15	empty	
8	yellow and fluorescent	
20	empty	
8	fluorescent	Section I
35	empty	
8	orange	Section II
50	empty	
70	fluorescent	Section III

Curves of all the fluorescent zones were taken in the Beckman spectrophotometer but only the solutions from Sections I and III showed a characteristic spectrum. The curves gave almost identical positions of the maxima and minima (corresponding to phytofluene) and they were estimated together using published values of $E^{1\%}_{1cm.}$ for phytofluene (72). The total amount estimated was 0.52 mg. for 920 g. or 0.6 mg. per kg. of fresh blossoms. Section II gave maxima at 478, 450 mμ and was estimated as β-carotene using the $E^{mol.}_{1cm.}$ value of 13.9×10^4. Total β-carotene was 0.05 mg. or 0.06 mg. per kg. of fresh blossoms.

B. ISOLATION OF PHYTOFLUENOL FROM TOMATO PASTE

30 kg. of commercial canned tomato paste (Campania - West Coast Packing Corp., Long Beach, Calif.) was the starting material. Each 3 kg - portion was shaken with 4 l. of 99% methanol for 20 mins. and filtered through a cloth in a basket centrifuge. The aqueous alcohol filtrate from each fraction was extracted with 1 l. of petroleum ether and the aqueous phase was then discarded. The partially dehydrated paste was worked up in 6 kg.-portions. Each portion was shaken for 20 mins. with 4 l. of methanol and filtered through a cloth in the basket centrifuge. The filtrate from each 6 kg.-portion was extracted with 1 l. of petroleum ether by adding water cautiously and swirling. If necessary the aqueous layer was extracted once more with 1 l. of petroleum ether (this was done if a small test indicated that fluorescent material would pass into the ligroin phase). The aqueous phase was then discarded. The partially dehydrated paste was worked up in 6 kg.-portions by shaking each portion with 3 l. of methanol + 3 l. of petroleum and filtering as above. This extraction was repeated 3 times for each portion, after which the fluorescence of the ligroin extract was very slight. The combined petroleum ether-methanol fraction was poured into 20 l. separatory funnels, water was added cautiously and the fluorescent material was transferred to the petroleum ether phase. The total ligroin solution was washed free of methanol, dried and filtered. The filtered solution was concentrated in vacuo (45°) to a total volume of 3 l. This concentrate was saponified overnight with 20% methanolic potassium hydroxide. After washing the petroleum ether solution free of methanol and alkali the precipitate of gummy crystals was filtered off. This precipitate was redissolved in

benzene and recrystallized giving crude lycopene. The mother liquors of crystallization were evaporated to dryness, redissolved in petroleum ether and added to the bulk of the saponified solution. This total solution was filtered through a 7 cm. layer of celite in a sintered glass funnel (12 cm. diameter) to get rid of gummy material which would interfere with chromatography. The celite was washed with petroleum ether until the carotenoids and fluorescent materials had passed through. (Some fluorescent material is adsorbed on the celite, but is discarded). The ligroin solution (5.4 l.) was chromatographed on ten columns (Sierra lime - Celite 3 : 1) and developed with 10% acetone in petroleum ether. Each column showed the following sequence (Figures on left denote width of zones in mm.):

35	buff, fluorescent	
22	orange-brown	
15	orange	
45	red, lycopene	
37	orange-red, neolycopene A	
10	yellow-orange	Section I, fluorescent
7	red-orange, contains γ-carotene	
12	yellow	
35	orange, β-carotene	
50	mixed orange and yellow zones, fluorescent	

Phytofluenol was not well separated at this stage and Section I included the lower half of the neolycopene A zone and the upper half of the 12 mm. yellow zone. The combined petroleum ether solutions from

sections I were rechromatographed on four columns and developed with 15% acetone in petroleum ether. The following sequence was observed from top to bottom:

7	colorless	
13	red	
25	red, lycopene	
48	orange, neolycopene A	
6	yellow	
17	pale orange	Section II,
25	yellow	fluorescent
25	red-orange	
40	yellow	
10	orange	
30	mixed zones	

The petroleum ether solution from the combined Sections II (containing the phytofluenol) was chromatographed on eight columns and developed with 15% acetone in petroleum ether giving the following sequence:

20	colorless
40	red, lycopene
50	orange, neolycopene A
30	pale orange
25	orange
30	yellow, phytofluenol
25	orange
60	colorless

The phytofluenol zones were combined, and their petroleum ether solution was chromatographed on one column (alumina-Celite, 4 : 1) and developed with 30% acetone in petroleum ether. The main, fluorescent zone was eluted, transferred to petroleum ether, washed and dried. An estimate of the amount (12 mg.) was made using values of $E^{1\%}_{1cm.}$ published for phytofluene (54). Evaporation to dryness gave a slightly yellow oil and attempts to crystallize it failed. Best values obtained with weighed samples gave $E^{1\%}_{1cm.}$ = 410. If the molecular weight of phytofluenol is greater than phytofluene by one oxygen atom (i.e. $C_{40}H_{64}O$) this would give a purity of 35% for the sample. An attempt was made to prepare an acetate by dissolving 1 mg. of phytofluenol in a few drops of pyridine and adding 1 drop of acetyl chloride. Subsequent chromatography and tests of the fluorescent material indicated no change in partition coefficient and adsorption behavior before and after treatment with methanolic potassium hydroxide. Partition coefficients between hexane - 83% (v/v) ethanol, and hexane - 95% methanol were carried out in the following manner. A solution of phytofluenol in hexane was mixed with a roughly equivalent volume of the aqueous methanol (or ethanol) and shaken in a separatory funnel. After the phases separated, equal volumes of the upper (U) and the lower (L) phases were taken. Hexane was added to L; then both U and L were washed free of alcohol and dried. The filtered solutions of U and L (in hexane) were diluted to equal volume and the extinction values determined in the Beckman spectrophotometer at the main maximum. Partition coefficient = C (upper) / C (lower) = E (upper) / E (lower). Values for phytofluenol obtained are 2.2 (hexane-95% methanol) and 6.0 (hexane - 83% ethanol).

III. SYNTHESIS OF STEREOISOMERIC DIPHENYLBUTADIENES

Light Sources and Conditions of Illumination - Chromatograms were inspected in the light of a General Electric Purple X bulb using a moulded Corning light filter No. 5840, 0.25 inch thick; "Mineralight Q31" was less satisfactory due to its different light filter. Iodine catalysis (in hexane solution) was carried out using amounts of iodine equal to 5% of the weight of dissolved material and illuminating for 10 to 20 mins. Under the same conditions benzene solutions should be illuminated for an hour. For artificial illumination of iodine-free solutions a 250-watt Mazda clear projection lamp bulb (Code -250 T 14/3 -120V) encased in a water jacket used (distance from filament to center of Pyrex volumetric flask, 10 cm.). Transparent quartz test-tubes were found satisfactory for insolation experiments (end temperature, 20-25°).

Thermal Treatment - Refluxing of hexane solutions (5-20 mg. per liter) was carried out in darkness, in a slow steam of carbon dioxide. In the melt experiments, 4-12 mg. weighed samples, in evacuated and sealed pyrex capillary tubes, were submerged in a dibutyl phthalate bath (205-210°), in darkness. After ten minutes the tubes were rapidly cooled in water and their contents examined without delay.

A. trans-Diphenylbutenine

$C_6H_5CH{=}CHC{\equiv}CC_6H_5$, was prepared (in a crude state) from phenylacetylene-copper according to Straus (47). Purification: a solution of 8.5 g. of brownish, crude crystals in 100 ml. of petroleum ether was developed with the same solvent on a silicic acid column, 22 x 4.8 cm. (The figures on the left designate thickness of the zones in mm.; fl. means fluorescence or fluorescent in ultraviolet light):

30 several brown zones (in daylight)
30 white fl. (1 g. oil)
7 brown (in daylight)
8 interzone, no fl.
50 white fl. (1 g. oil)
80 column-fl. quenched: main product
Filtrate: no fl.

The main zone was cut out, eluted with ethanol, transferred into petroleum ether, dried with sodium sulfate and completely evaporated in vacuo. A solution of the slightly colored crystals (5.5 g.) in 50 ml. of hot methanol deposited 3 g. of white needles in the cold room; m.p. 96°, cor. An additional 1 g. can be obtained by chromatographing the mother liquor as above.

B. cis-trans-Diphenylbutadiene from trans-Diphenylbutenine.

In an all-glass apparatus (with mercury-sealed stirrer) the solution of 3 g. of the butenine in 250 ml. of alcohol was refluxed in darkness with 2 g. of zinc-copper dust (from copper sulfate and zinc, washed with ethanol) and another 2 g.-portion of the metals was introduced after thirty hours. The sharp extinction maximum (originally at 306 mμ, in petroleum ether) gradually disappeared and, after one hundred and ten hours of refluxing, the smooth maximum of cis-trans-diphenylbutadiene was observed in a small sample. Then 250 ml. of petroleum ether and 1 liter of water were added to the filtered liquid; the aqueous layer was re-extracted and the total petroleum ether solution was repeatedly washed and dried. The following chromatogram, obtained with the same solvent, on a 28 x 8 cm. alumina column, refers to one third of the crude solution:

30 several, partly fl. zones

25 blue fl.: trans-trans-diphenylbutadiene

140 fl. of alumina quenched: crude cis-trans

When the bottom of the lowest zone was 7 cm. from the end of the column, the quenched section was cut out in two halves of which the upper one contained unchanged butenine (its extinction curve was not influenced by iodine). The bottom half of the quenched zone showed the spectroscopic character of cis-trans-diphenylbutadiene, and a sample yielded (after iodine catalysis in light) the fine structure of the trans-trans form. This fraction was rechromatographed as above.

In order to eliminate the last impurities, especially for optical

observations, 3 mg. of the cis-trans compound (in 3 ml. of petroleum ether) was adsorbed on a 20 x 1.9 cm. column (alumina) and washed in the same solvent. Each 10-ml. portion of the (non-fluorescing) filtrate was tested as follows: A few drops were catalyzed with one drop of iodine solution (0.3 mg./ml.) and the test-tube exposed to the 250-watt lamp from a 10 cm. distance for one to two minutes. If the sample showed fluorescence in ultraviolet light, the fraction was kept, otherwise rejected. This test was positive between the fractions 17-29. Fractions 18-28 were found spectroscopically to contain pure cis-trans compound; 3 g. of the butenine yielded 0.75 g. of the purest product.

The molecular extinction coefficients were given earlier (73).

Anal. Calcd. for $C_{16}H_{14}$: C, 93.16; H, 6.84. Found: C, 92.72, 92.89; H, 6.84, 6.88.

C. cis-cis-Diphenylbutadiene

cis-cis-Diphenylbutadiene was prepared by catalytic reduction of diphenyldiacetylene, $C_6H_5C{\equiv}CC{\equiv}CC_6H_5$, by Kelber and Schwarz (26) as well as by Ott and Schroter (41) in yields of 8-32%, depending on the catalyst. Although our yield remained between these limits (for example, 24%), we recommend the following isolation which is based on spectroscopic control of the reduction process and chromatographic purification of the product. During the reaction the triple bonds are reduced not only to double, but in part also to single bonds. These competing processes can be followed by the changing extinction curve. The sharp and high peaks of diphenyldiacetylene at 306 and 326 mμ. (in hexane) gradually disappear and the much lower, smooth maximum of cis-cis-diphenylbutadiene at 300 mμ. then makes the main contribution to the curve. A satisfactory amount of the latter compound is clearly indicated if a small sample (diluted with hexane) is examined in the spectrophotometer before and after iodine catalysis (in light). The new maximum (now at 328 mμ.) whould be at least 1.5 times higher than $E_{max.}$ was before this catalytic treatment.

The following operations should be carried out in dim light. A solution of 1 g. of diphenyldiacetylene in 100 ml. of 95% ethanol was shaken with 0.25 g. of palladium-barium sulfate (40) until roughly 350 ml. of hydrogen was taken up within one quarter to one half hour. After filtration, the material was transferred with water into petroleum ether and the aqueous phase re-extracted. The combined petroleum ether

solution, after washing, drying and concentrating to 50 ml., was developed on the alumina column (30 x 7.5 cm.) with about 2 liters of the same solvent:

6	dark (in uviol. light)
8	greenish fl.
20	blue fl.: trans-trans-diphenylbutadiene
16	non-fl. interzone
60	column-fl. quenched: cis-trans-diphenylbutadiene
26	interzone (borders blurred)
50	column-fl. quenched: cis-cis-diphenylbutadiene
110	empty section

The location of the cis-cis compound possibly could be improved by using a strongly fluorescent column as proposed by Sease (45) and Brockmann(3).

The cis-cis zone was cut out, eluted with ethanol, transferred into petroleum ether, washed, dried and completely evaporated in vacuo. The oily residue was then transferred into a small centrifuge tube and dissolved in a minimum amount of warm 95% ethanol. Crystallization was observed at room temperature, whereupon the tube was kept at 4° overnight. Yield, after recrystallization, was 243 mg. of cis-cis-diphenylbutadiene which showed distinctly different crystal forms from those of the trans-trans isomer. The extinction coefficients are given in Table XII.

Anal. Calcd. for $C_{16}H_{14}$: C, 93.16; H, 6.84. Found: C, 93.13; H, 7.17.

Table XII

Molecular Extinction Coefficients of cis-cis-Diphenylbutadiene at the Maxima (italicized) and Minima

	cis-cis form		Mixture of Stereoisomers upon iodine catalysis	
Solvent	mμ	$E^{mol.}_{1cm.}$ x 10^{-4}	mμ	$E^{mol.}_{1cm.}$ x 10^{-4}
Hexane	*299-300*	2.96	*344-345*	3.40
	251	0.51	340	3.35
			328	5.36
			318-319	4.50
			315-316	4.56
			255	0.20
			230-231	1.36
Benzene	*306*	2.67	*352-353*	3.17
			347	2.95
			334-335	4.85
			325-326	4.00
			321-322	4.05

Small-Scale Separation of the Three Stereoisomeric Diphenylbutadienes - A solution which contained 1 mg. of each isomer in 10 ml. of petroleum ether was developed with the same solvent on alumina (20 x 2 cm.); the developer was forced through the column by nitrogen pressure in order to avoid partial evaporation of the filtrate.

The fluorescent trans-trans compound remained near the top. Small samples of each fraction collected from the filtrate were submitted to the iodine-catalysis and fluorescence test as described. The first 120 ml. of the flow were found to be free of substance; subsequent 5-ml. fractions were tested with the following result ("cis-cis" or "cis-trans" refer to the configuration before iodine catalysis):

No.		
	1-7	strong fl.: cis-cis
	8-9	weak fl.: cis-cis
	10-12	almost no fl.: traces
	13-21	very strong fl.: cis-trans
	22-26	strong fl.: cis-trans
	27-30	weak fl.: cis-trans
	31	no fl.

The fractions were also tested spectroscopically.

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Propositions submitted by J. H. Pinckard

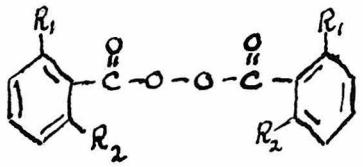
1. Since it has been proposed that steric hindrance of certain double bonds in carotenoids will prevent a cis configuration at this bond, and since this is based on the concept of coplanarity, it would be of value to know the resonance energies of carotenoids, and the difference in energies between stereoisomeric forms of carotenoids. The determination of these energies would be possible through (a) Heats of hydrogenation, and (b) Heats of combustion.

   L. Pauling, Fortschr. Chem. Organ. Naturstoffe 3, 203 (1939).

   G. B. Kistiakowsky et al. J. A. C. S. 58, 146, (1936)

   R. J. Corruccini and E. C. Gilbert, J. A. C. S. 61, 2925 (1939)

2. In the conversion of provitamins A to vitamin A, in biological systems, there is a specific attack on the central double bond of the molecule. Since the arrangement of methyl groups around this double bond is different from that of other double bonds, I propose that properly substituted diacyl peroxides of the type



   would attack this central double bond in preference to others.

3. Since it is difficult to obtain poly-cis forms of polyenes, either by direct synthesis or by isolation from natural sources, the two following methods of producing such forms should be investigated:

a. Application of very high pressures with moderate heating.

b. Irradiation with monochromatic light, or light which is suitably filtered to eliminate certain frequencies which would promote re-isomerization.

4. I propose that a study of the effect of ethylene on the ripening processes in some fruits should be carried out with C^{14} labelled ethylene, followed by isolation of some compounds from the fruit, in order to follow the course of reactions taking place during ripening.

5. The effect of compounds with halogen-like properties, such as thiocyanogen, in producing cis-trans isomerization, should be studied.

6. Since carotenoid accumulation occurs in such animal tissues as the corpus luteum and fat bodies, it would be of interest, e.g. in the frog, to test the viability of sperm or eggs in carotenoid depleted animals.

L. Zechmeister, from Ergebnisse der Physiologie, biologischen Chemie und experimentellen Pharmakologie, (1937).

7. The use of more or less homogeneous protein materials, such as casein, albumin, etc., as adsorbents for chromatographic separation of carotenoids and other substances, should be tested.

8. Some acetylenic compounds yield cis isomers of the polyene produced by reduction. It would be of interest to synthesize diaryl-polyacetylenes of the type $AR-(C{\equiv}C)_n-AR$, where n is greater than two, and determine whether stable cis isomers of $AR-(CH{=}CH)_n-AR$ are produced by reduction. This would be of interest, since no stable cis isomers of higher polyenes of this type are known.

R. Kuhn and A. Winterstein, Helv. Chim. Acta 11, 144 (1928)

9. Since some fresh-water fish accumulate vitamin A_2 in the liver, it would be of value to carry out feeding experiments, using different carotenoids, to see whether there is a relation between the kind of carotenoid and vitamin produced.

R. A. Morton, Nature 153, 69, 405 (1944).

10. It is difficult for students to get an idea of the research program carried out by various groups in the chemistry department. I propose that bound volumes be prepared, containing the original papers published by members of this division, and that these volumes be placed in the department library.