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Spectral Characteristics and Configuration of Some Stereoisomeric Carotenoids Including Prolycopene and Pro-γ-carotene

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As is well known,[1] a natural, all-*trans* carotenoid is partially converted by heat or iodine into a mixture of its *cis–trans* isomers. Simultaneously a decrease in the intensities and wave lengths of the extinction maxima takes place in the visible spectral region. These observations are in accordance with the theoretical conclusion[2,3] that among all stereoisomers of a polyene the all-*trans* form, possessing the extended coplanar structure, must have the greatest color intensity.

It was recently shown,[4] however, that the bending of the molecule does not necessarily involve a decrease in the extinction in every spectral region. On the contrary, such a spatial change, brought about, *e. g.*, by iodine catalysis, causes the appearance of a new marked maximum which we call the "*cis*-peak." The latter is located somewhere within the region 320 to 380 mμ, *e. g.*, at 339.5 mμ for β-carotene in hexane. The distance between this peak and the longest wave length maximum is approximately constant, 142 mμ for all C_{40}-carotenoids tested so far.

It seemed probable that the different stereoisomers present in the complicated equilibrium mixture would contribute unequally to the total effect observed, the contribution of the sterically symmetrical all-*trans* form being practically zero. This assumption was first verified for the β-carotene stereoisomeric set,[4] and in the present paper we report more extensive pertinent results for the γ-carotene set and especially for the lycopene set.

In the investigation of β-carotene it was found[5] that neo-β-carotene U, which is adsorbed above natural β-carotene in the Tswett column, shows a considerably smaller *cis*-peak than the equimolecular solution of the stereoisomeric mixture obtained with iodine. In contrast, the isomers adsorbed below natural β-carotene possess a much higher *cis*-peak than the mixture.

In order to obtain a broader experimental basis for theoretical work, it is necessary to investigate whether a stepwise *trans* → *cis* change of an all-*trans* conjugated system will raise continuously the *cis*-peak, or whether the peak will reach a maximum intensity and then decrease again as the number of *cis* double bonds in the molecule increases. Particularly suitable materials for such an investigation are offered by nature in the form of pro-γ-carotene, $C_{40}H_{56}$, and prolycopene, $C_{40}H_{56}$, which have been isolated from several fruits[6] but not yet obtained artificially.

The positions of the maxima of these isomers dissolved in petroleum ether differ from those of the respective all-*trans* forms by 31 and 35 mμ, respectively. Since the difference between all-*trans* carotenoid hydrocarbons and their spectroscopically closest stereoisomers is about 4 to 6 mμ (corresponding to one *trans–cis* change), it is evident that the pro-compounds must possess a considerable number of *cis*-bonds. This statement would remain valid even if the unit increment should increase with progressing *trans–cis* rotations.

It has been pointed out[2] that, because of steric conflicts, only five of the eleven conjugated double bonds are available for the *cis*-configuration in β-carotene, six in γ-carotene, and seven in lycopene (see the formulas).

The pro-carotenoids cannot, however, contain these numbers of *cis*-bonds, for the following reason. If crystals of either prolycopene or pro-γ-carotene are melted and then chromatographed, there appears in the lowest section of the Tswett column a minor isomer which has its maxima at still shorter wave lengths than the corresponding pro-compound. It is, therefore, probable that these minor zones contain the all-*cis* isomers (in the restricted steric sense mentioned, with six or seven *cis*-bonds, respectively), and that only five *cis*-bonds are present in pro-γ-carotene and six in prolycopene.

(1) For literature see our recent paper, A. Polgár and L. Zechmeister, THIS JOURNAL, **64**, 1856 (1942).

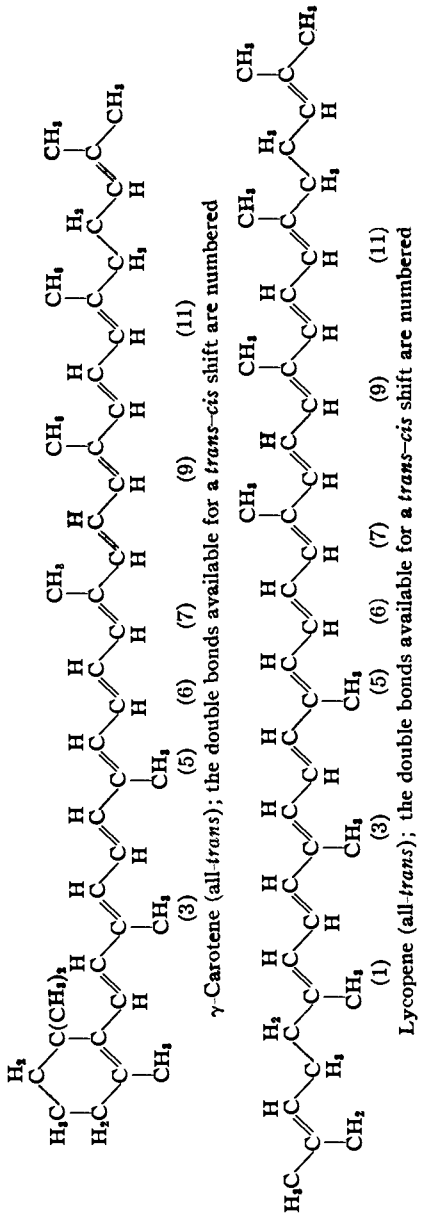
(2) L. Pauling, *Fortschr. Chem. organ. Naturstoffe*, **3**, 203 (1939).

(3) R. S. Mulliken, *J. Chem. Phys.*, **7**, 364 (1939); *Rev. Modern Phys.*, **14**, 265 (1942).

(4) L. Zechmeister and A. Polgár, THIS JOURNAL, **65**, 1522 (1943).

(5) Reference 4, Fig. 4.

(6) L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Natl. Acad. Sci.*, **27**, 468 (1941); A. L. LeRosen and L. Zechmeister, THIS JOURNAL, **64**, 1075 (1942); L. Zechmeister and W. A. Schroeder, *Science*, **94**, 609 (1941); THIS JOURNAL, **64**, 1173 (1942); *J. Biol. Chem.*, **144**, 315 (1942); L. Zechmeister and R. B. Escue, *ibid.*, **144**, 321 (1942).



γ-Carotene (all-*trans*); the double bonds available for a *trans–cis* shift are numbered (3) (5) (6) (7) (9) (11)

Lycopene (all-*trans*); the double bonds available for a *trans–cis* shift are numbered (1) (3) (5) (6) (7) (9) (11)

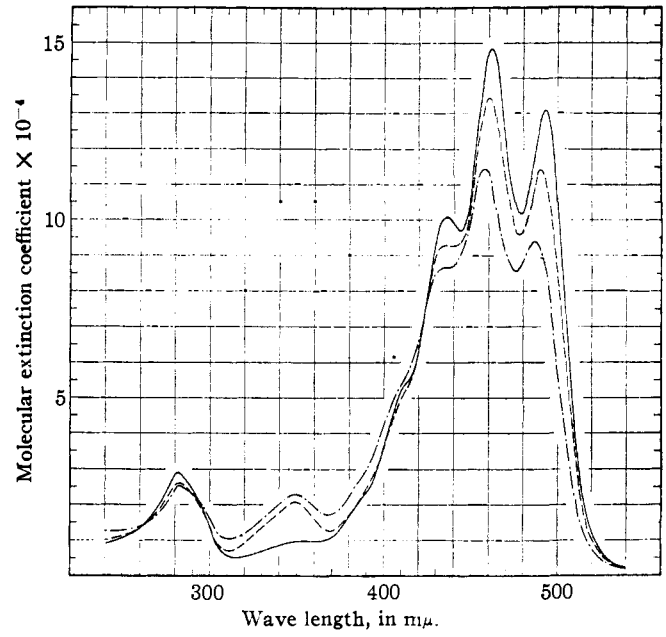


Fig. 1.—Molecular extinction curves of γ-carotene in hexane: ———, all-*trans* form; - - -, after refluxing in the dark for forty-five minutes; —·—, after iodine catalysis at room temperature.

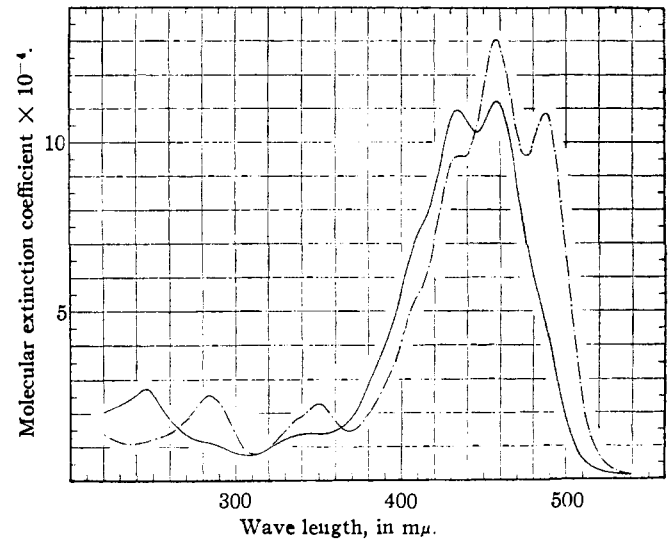


Fig. 2.—Molecular extinction curves of pro-γ-carotene in hexane: ———, without iodine, —·—, after iodine catalysis.

A comparison of Figs. 1 and 2 shows that in the visible region the extinction curve of pro-γ-carotene is much flatter than that of its all-*trans* isomer. The two have, however, an important spectral characteristic in common, namely, the absence of a *cis*-peak. Such a peak appears at 350 mμ if iodine is added to either of the two solutions; and simultaneously the great difference between the curves of γ-carotene and pro-γ-carotene in the visible region disappears.

We were able to carry out a more detailed investigation in the case of the lycopene set (Figs. 3–6) in which the extinction curves of the stereoisomers listed in Table I were taken.

As shown in Figs. 3–5, the curves of No. (1), (3), (4), and (5) practically do not include *cis*-peaks while neolycopene A, which optically and chromatographically stands closest to natural (all-*trans*-) lycopene, shows the highest peak so far

TABLE I

IMPORTANT STEREOISOMERS OF THE LYCOPENE SET

No.	Name	Visible maxima in petroleum ether, (mμ) (b. p. 60–70°)		Maximum height of the molecular extinction curve in hexane
(1)	All-*trans*-lycopene	504.5	473.5	18.6×10^4
(2)	Neolycopene A	500	470	12.2
(3)	An unnamed crystalline isomer	476	444	10.2
(4)	Prolycopene	470–5	445	10.2
(5)	All-*cis*-lycopene	466	439	8.7

observed in this set (Fig. 6). Evidently this isomer,[7] which is present in substantial quantities, is mainly responsible for the *cis*-peak effect given by the total equilibrium mixture.

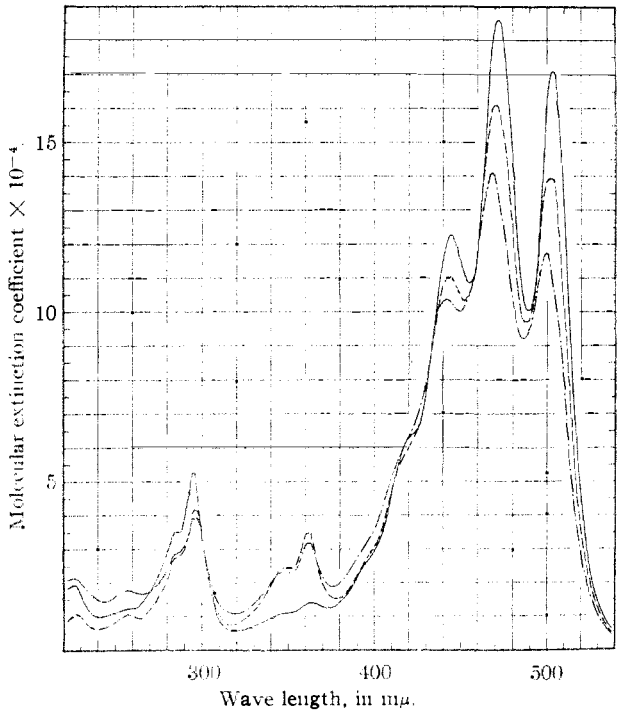


Fig. 3.—Molecular extinction curves of lycopene in hexane: ———, all-*trans* form; – – – –, after refluxing in the dark for forty-five minutes; ····, after iodine catalysis at room temperature

This statement, of course, does not mean that others of the 72 isomers may not have *cis*-peaks comparable with that of neolycopene A. Chromatography so far has not revealed more than a dozen stereoisomers for any carotenoid, which constitute only a fraction of those theoretically expected. In this connection mention may be made of the great lability of certain pigments which are observed in adsorbates. It was noted, for example, that some β-carotene isomers can hardly stand even elution and transfer into another solvent at room temperature without steric alteration.[1] It is also interesting that different isomers vary with respect to relative ease of isomerization under the influence of different agents or conditions. For example, refluxing in hexane substantially modifies the curves of the all-*trans* forms of γ-carotene and lycopene (Figs. 1 and 3), whereas no such alterations were observed in parallel experiments with the corresponding pro-carotenoids. (The thermal isomerization curves of pro-γ-carotene and prolycopene are not given in Figs. 2 and 4, since they are practically identical with the curves taken without refluxing.) On the other hand, the pro-compounds, like the all-*trans* forms, are extremely sensitive to iodine.

Pro-γ-carotene and prolycopene are photosensitive even in the absence of catalysts. Their flat extinction curves are converted by short insolation into the much more complicated curves of steric mixtures (Figs. 7 and 8), the composition of which will be investigated later. Although some destruction occurs under the influence of light, this was limited in amount under the conditions applied. The extinction values are much higher after insolation than before.

On the basis of all available experimental information and of the theoretical arguments given below, we believe that the spectroscopic feature termed "*cis*-peak" is characteristic for those stereoisomers of carotenoids in which the molecules have a bent shape such as results from the assumption of the *cis*-configuration by a double bond at or near the center of the chromophore. It is understandable from arguments given below why both the all-*trans*- and the all-*cis*-configurations are spectroscopically ineffective in the *cis*-peak region. If pro-γ-carotene and prolycopene have the *cis*-configuration about all of the stereochemically available double bonds except one, it is not unreasonable to assume that this unique bond is the one in the center of the chromophore. Such a configuration would not produce a *cis*-peak; in this respect the naturally occurring pro-carotenoids behave like the all-*cis* compounds.

We accordingly conclude, answering the question posed earlier, that the intensity of the *cis*-peak is not determined simply by the number of

(7) L. Zechmeister and P. Tuzson, *Biochem. J.*, **32**, 1305 (1938); *Ber.*, **72**, 1340 (1939).

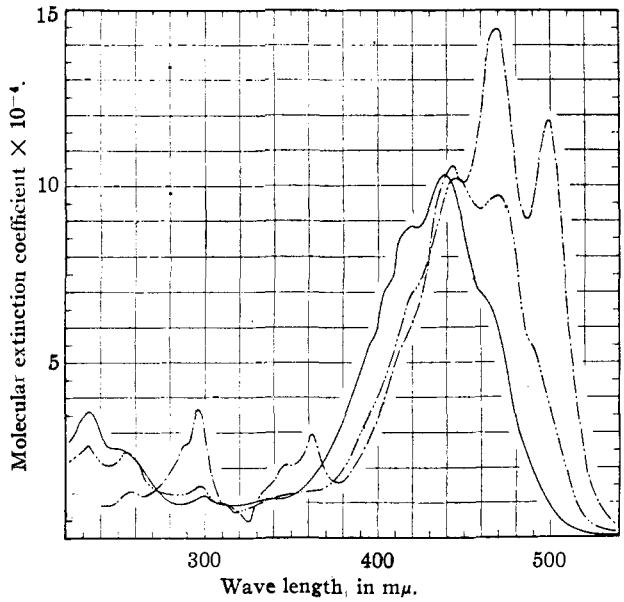


Fig. 4.—Molecular extinction curves of prolycopene and of a crystallizable isomer in hexane: ———, prolycopene without iodine; —·—, prolycopene after iodine catalysis; ···—···—, crystallizable stereoisomer (first band at 476 mμ., visually) without iodine.

cis double bonds, but depends on the detailed structure of the molecule; the nature of this dependency is discussed in the theoretical part of the present paper.

As a typical example of stereoisomers showing a very considerable *cis*-peak effect, neolycopene A was mentioned. Since its extinction in the region under discussion is much higher than that of the equilibrium mixture, on addition of iodine to solutions of pure neolycopene A a very considerable decrease of the peak is observed (Fig. 6).

On the basis of their spectral changes which are observed on addition of iodine, the following types of phenomena may be listed:

all-*trans*-lycopene: increase of the extinction in the *cis*-peak region, and decrease in the visible region;

neolycopene A: decrease in the *cis*-peak region and increase in the visible region;

prolycopene and all-*cis*-lycopene: increase in both regions.

Such changes are also observed in other sets of carotenoid stereoisomers now under investigation.

Theoretical Discussion

The development in recent years of the quantum mechanical theory of the color of organic pigments[8,9] has made it possible to derive from spectrophotometric data for the carotenoid isomers valuable information about the configurations of their molecules. The following discussion, which is based in part on earlier treatments,[2,10,11] is designed to present the qualitative interpretation of the phenomena in a simple way. We plan to postpone the quantitative comparison of experiment and theory until a larger body of experimental results has been gathered and external conditions permit making detailed calculations. The following discussion is applicable in its general results to any set of carotenoid stereoisomers. For the sake of precision the argument is given in detail for the special case of a symmetrical molecule with an odd number of conjugated double bonds (lycopene).

The observed absorption spectra of the carotenoids correspond to transitions from the normal electronic state to three excited electronic states (Fig. 9). The main absorption band, in the region (for lycopene in petroleum ether, for example) near 470 mμ, results from the transition 0 → 1, the "*cis*-peak"

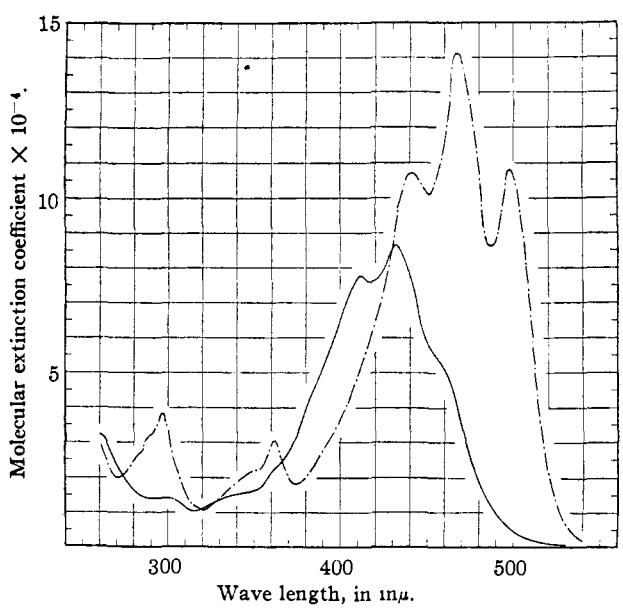


Fig. 5.—Molecular extinction curves of all-*cis*-lycopene in hexane: ———, all-*cis*-lycopene without iodine; —·—, after iodine catalysis.

(8) L. Pauling, in Gilman's "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1938, p. 1888; *Proc. Natl. Acad. Sci.*, **25**, 577 (1939).

(9) R. S. Mulliken, *J. Chem. Phys.*, **7**, 14, 20, 121, 339, 353 (1939).

(10) R. S. Mulliken, *ibid.*, **7**, 364 (1939).

(11) G. N. Lewis and M. Calvin, *Chem. Rev.*, **25**, 273 (1939).

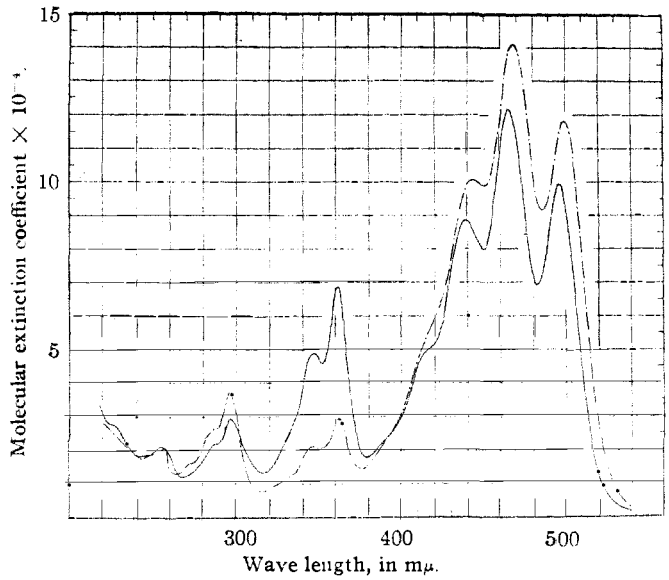
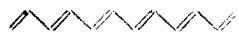
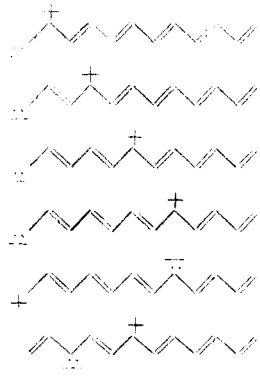


Fig. 6.—Molecular extinction curves of neolycopene A: ———, without iodine; – – – –, on iodine catalysis.

from 0 → 2, and the third band, near 270 mμ, from 0 → 3. These bands are broad and show a more or less pronounced vibrational fine-structure. In the language of the valence-bond theory, these electronic levels of the conjugated system may be discussed in terms of the conventional structure



for the molecule and a great number of ionic structures in which there is a separation of electric charge, such as



These structures do not correspond individually to the normal state and various excited states of the molecule; instead, the molecule in each particular state resonates among the structures. The conventional structure of alternating double and single bonds makes a most important contribution to the normal state, whereas the ionic structures contribute in the main to the excited states.

On carrying out the quantum mechanical calculations it is found that the three transitions 0 → 1, 0 → 2, and 0 → 3 correspond to oscillation of electric charge along the unsaturated chain in the ways shown in Fig. 10.

Following Lewis and Calvin[11] we may compare these with the classical modes of vibration of mobile "unsaturation" electrons of the conjugated system along the chain. The simplest of these classical modes of vibration is that in which electrons tend to concentrate first near one end and then near the other end of the chain; this simple oscillation would, according to classical electromagnetic theory, result from the absorption of light of the proper frequency because of interaction of the electric vector of the light and the regularly reversing electric dipole moment of the molecule. This mode of oscillation of the charge in the molecule corresponds to the transition 0 → 1.

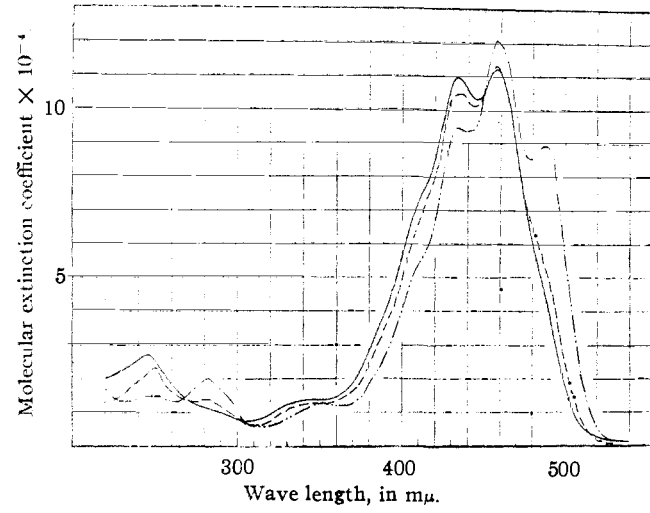


Fig. 7.—Molecular extinction curves of pro-γ-carotene before and after insolation: ———, before insolation; — — —, after five minute insolation; — · — · —, after thirty minute insolation.

The next mode of oscillation of the electrons is from the two ends of the conjugated system toward the middle and from the middle toward the two ends. This corresponds to the transition 0 → 2. The third, corresponding to the transition 0 → 3, involves concentration of the electrons alternately

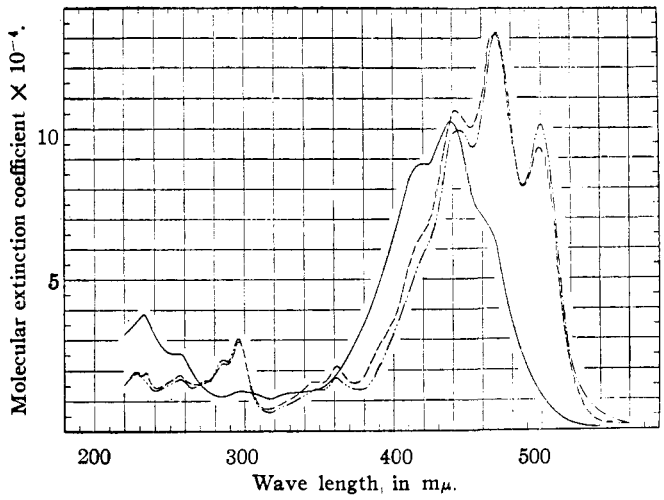


Fig. 8.—Molecular extinction curves of prolycopene before and after insolation: ———, before insolation; —··—··, after twenty minute insolation; — — —, after sixty minute insolation.

in the first and third and the second and fourth quarters of the conjugated system.

We have assumed that the observed isomerization of the carotenoids is *cis–trans* isomerization about certain double bonds,[12] to wit, those which do not have methyl groups on the adjacent carbon atoms,[2] and that the other bonds have essentially *trans*-configurations. Some of the possible isomers of this sort are shown in Fig. 11.

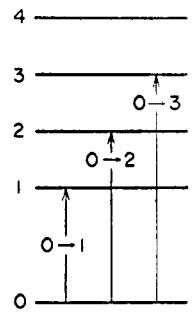


Fig. 9.—Electronic energy levels for a carotenoid molecule, with the transitions indicated corresponding to the three main absorption regions (0 ⟶ 1, fundamental absorption region; 0 ⟶ 2, "*cis*-peak" region or first overtone; 0 ⟶ 3, second overtone).

The intensity of an absorption band is proportional to the square of the corresponding dipole moment, and hence essentially to the square of the length of the system.[2,3] The maximum intensity of the fundamental band would be shown by the all-*trans* molecule (I, Fig. 11); the fact that for each carotenoid so far investigated the ordinary natural isomer shows stronger absorption in the region of the fundamental band than other isomers supports the previously accepted assignment of the all-*trans* configuration to these compounds.

All stereoisomers of lycopene which have a vertical plane of symmetry, such as II and V in Fig. 11, have a distance between the ends of the conjugated system smaller than the all-*trans* isomer by the factor cos α, with $\alpha = 27°22'$ if the carbon bond angle along the chain is 125°16′. The fundamental spectral band for these isomers should then be approximately 80% as strong as for the all-*trans* form, since $\cos^2\alpha = 0.80$. Reference to Figs. 2, 4, 5 and 6 shows that several isomers, including the pro-carotenoids and neolycopene A, have about the expected intensity ratio with the all-*trans* isomer.[13]

The intensities for all isomers permitted by the above postulates should lie between these extremes. The fact that this agrees with observation provides support of our assumption that only certain double bonds can have the *cis*-configuration, and that the other double bonds and the single bonds of the conjugated system have essentially the *trans*-configuration.

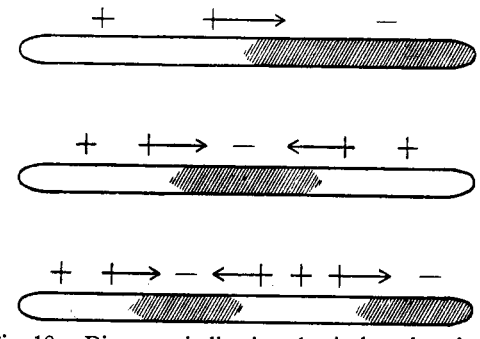


Fig. 10.—Diagrams indicating classical modes of vibration of mobile "unsaturation" electrons of the conjugated system along the chain. The three modes shown correspond to the three main regions of absorption of light by carotenoids (see for example Fig. 6).

If the single bonds also were *cis*, isomers might exist with very small absorption in the fundamental region.

The nature of the 0 ⟶ 2 oscillation is such that it gives rise to no dipole moment and hence to no absorption band for the all-*trans* molecule or any other molecule, such as IV (Fig. 11), with a center of symmetry.[14] The molecule which is *cis* at the central double bond (II, Fig. 11), has, however, a dipole moment for the transition 0 ⟶ 2, because of its shape; this dipole moment is perpendicular

(12) L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1340 (1939); L. Zechmeister, L. Cholnoky and A. Polgár, *ibid.*, **72**, 1678, 2039 (1939).

(13) In comparing intensities essentially the ratio of areas rather than of peaks is to be taken.

(14) The intensity of the 0 ⟶ 2 transition was discussed by Mulliken (ref. 3).

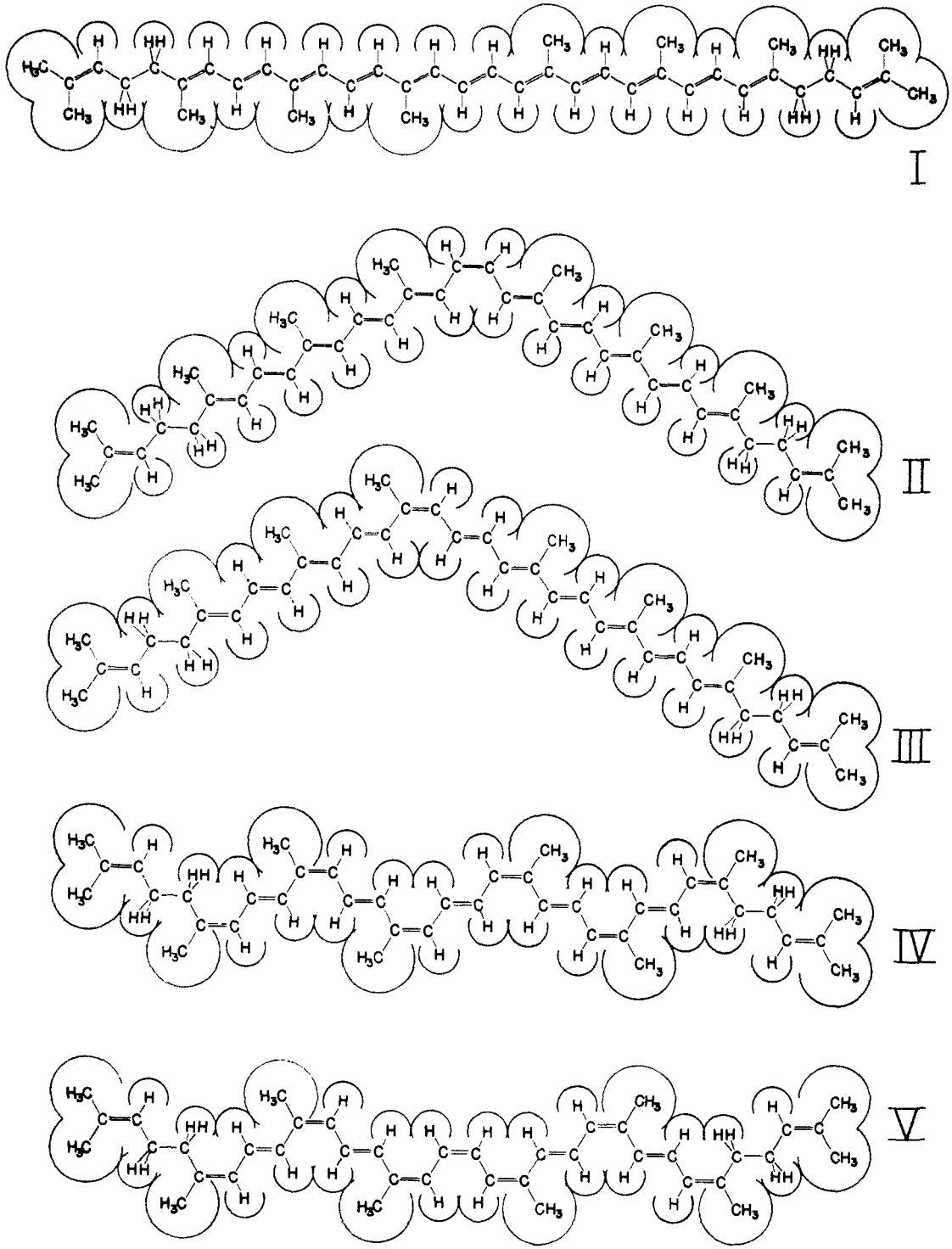


Fig. 11.—Some important stereoisomeric structures of the lycopene set, and the proposed correlation with observed isomers: I, all-*trans*-lycopene (ordinary lycopene); II, 6-*cis*-lycopene (neolycopene A); III, 5-*cis*-lycopene (not yet correlated with an observed isomer); IV, 1, 3, 5, 7, 9, 11-*cis*-lycopene (prolycopene); V, 1, 3, 5, 6, 7, 9, 11-*cis*-lycopene or all-*cis*-lycopene (isomer located below prolycopene in the chromatogram). The bond angles and the relative bond distances and van der Waals radii correspond approximately to the accepted values.

to the line between the ends of the conjugated system, instead of parallel to it. This isomer would show the strongest *cis*-peak of all. Certain other isomers, such as III (Fig. 11), would also have *cis*-peaks but of smaller intensity. As a rough approximation the intensity of the *cis*-peak can be taken 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. Accordingly only a few isomers can have *cis*-peaks approaching in intensity that of II.

The intensity of the third maximum, $0 \rightarrow 3$, may be expected to vary in rough proportionality to that of the fundamental. This is verified by experiment. Some deviation would be expected for molecules with two bends, for which the $0 \rightarrow 3$ peak would show a smaller drop below that of the all-*trans* form than the $0 \rightarrow 1$ band.

By use of intensities as well as wave lengths the identification of some isomers may be made with some confidence. Neolycopene A, which has a very high *cis*-peak, must surely be the isomer II (Fig. 11), *cis* about the central double bond only.

The assignment of structures to prolycopene and the "all-*cis*" isomer is based on the observed spectral shifts, which are about six or seven times as great as for the mono-*cis* isomers, relative to the all-*trans* form. Prolycopene was at first tentatively assigned the all-*cis* configuration[5]; after study of the isomer obtained from it by melting, this new isomer was assigned the all-*cis* configuration, and prolycopene the configuration with six *cis* double bonds[6] (IV, Fig. 11).

These assignments of structures may be somewhat inaccurate, since they are based on the assumption that each *cis* double bond causes a wave length shift of about 5 mμ, and it is probable that the shifts for the different double bonds may be somewhat larger or smaller than this. In particular is it probable that the spectral effect of the end double bonds of the conjugated system, 1 and 11 in lycopene, is small, and that the configuration about these bonds may well be *trans* in both prolycopene and the "all-*cis*" isomer.

The theory of conjugated systems permits some predictions to be made concerning the ease of thermal isomerization about different double bonds. Each of the double bonds in a conjugated system loses some of its double-bond character to the adjoining single bonds, the amount lost increasing from the ends toward the center of the system.[14a] Hence the central double bond has the smallest amount of double bond character, and accordingly may be predicted to undergo thermal *cis–trans* isomerization at the relatively lowest temperature. It is possible that this effect is enhanced by the difference in attached groups for the central bond and the other bonds:

$$-CH{=}CH- \textit{ versus } -CH{=}C(-CH_3)-$$

This argument is compatible with the ease of thermal isomerization, *e. g.*, of all-*cis*-lycopene to prolycopene and of all-*trans*-α-carotene, -β-carotene, -γ-carotene, -lycopene, etc, to isomers of the neolycopene A type (II, Fig. 11). Each of these changes involves only rotation about the central double bond.

It is of interest to consider the consequences of the postulate that the activation energy for *trans* $\rightarrow$ *cis* isomerization about the central double bond is less than for the other double bonds of the conjugated system. This leads to the classification of the 72 isomers of the lycopene set into 36 pairs, the members of each pair (*cis* and *trans*, respectively, about the central double bond, otherwise with the same configuration) being especially easily interconvertible, and the 6-*trans* isomer of each pair being more stable thermodynamically than its 6-*cis* analog. We deduce that for each pair the thermal isomerizations

$$6\text{-}trans \rightleftarrows 6\text{-}cis$$

(*e. g.*, all-*trans*-lycopene $\rightleftarrows$ neolycopene A or prolycopene $\rightleftarrows$ all-*cis*-lycopene) would occur readily at lower temperatures than isomerizations involving other double bonds; and, since the activation energies for forward and reverse reactions differ by the heat of reaction, the less stable isomer of each pair (the 6-*cis* isomer) should isomerize at lower temperatures than the more stable isomer (6-*trans*).

These predictions have so far been tested by experiment to only a small extent; it has been shown, for example, that thermal isomerization of all-*trans*-lycopene produces neolycopene A (6-*cis*-lycopene) preferentially to other isomers, as compared with iodine catalysis, and also that

(14a) This results from the fact that the amount of conjugation increases from the ends to the center of the system, as can be seen by consideration of the various resonating structures; thus of the various ionic structures of the sort shown above there are more which give single-bond character to the double bonds near the center of the system than to the double bonds near the end. For a quantitative treatment see, for example, C. A. Coulson, *Proc. Roy. Soc.* (London), **A169**, 413 (1939).

neolycopene A undergoes thermal isomerization to the all-*trans* form with great ease.

We anticipate that this ease of interconversion of the members of a 6-*cis*–*trans* pair will be used as the basis of a simple experimental method for identifying pairs among observed isomers, as a first step in assignment of structures.

Dr. V. Schomaker has pointed out to us that the ease of interconversion of *cis*–*trans* isomers is decreased by any influence which decreases the amount of conjugation in the conjugated chain, since this decrease in the amount of conjugation increases the amount of double-bond character of the double bonds. In particular, the presence of the *cis* configuration for double bonds in the system, which interferes with the conjugation and produces a spectral shift toward the violet, would make this *cis*–*trans* isomerization more difficult. This theoretical argument is substantiated by the fact that the procarotenoids, with several *cis* double bonds, undergo thermal isomerization less readily than the corresponding all-*trans* isomers.

It is of interest to ask why only about a dozen stereoisomers of, for example, lycopene, out of the 72 expected, appear on chromatographic analysis of the steroisomeric mixtures obtained by iodine catalysis, heating, etc. Experiments indicate that the equilibrium ratio mono-*cis*/all-*trans* at room temperature is of the order of magnitude of 0.1; if we assume that about the same change in free energy accompanies *trans*–*cis* isomerization about each double bond, the equilibrium ratio of amounts of isomers to that of the all-*trans* form would be 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001 and 0.0000001 for isomers with 1, 2, 3, 4, 5, 6 and 7 *cis* double bonds, respectively. The detection and isolation of isomers with more than three *cis* double bonds from an equilibrium mixture would accordingly be extremely difficult. It is possible that a steady-state mixture resulting, *e. g.*, from irradiation with light of suitable wave lengths would contain some of these isomers in greater amounts, permitting their isolation and identification. Another method, illustrated in this paper, involves the use as starting material of thermodynamically unstable isomers occurring in nature (prolycopene, pro-γ-carotene, etc.). Mixtures obtained by partial isomerization of such an isomer by various methods would contain various isomers in amounts determined by their rates of formation from the starting substance, which would depend on their structural relationships. We believe that it will be possible ultimately to separate and to identify all or most of the theoretically expected stereoisomers of some of the carotenoid sets.

Acknowledgment.—The authors are indebted to Professor A. J. Haagen-Smit and Dr. G. Oppenheimer for analyses including catalytic microhydrogenations.

Experimental

Methods.—The adsorbent used was calcium hydroxide (Shell Brand lime, chemical hydrate, 98% through 325 mesh). For chromatographic purposes the solvent used was petroleum ether ("Skellysolve B," b. p. 60–70°). The solvent for photometric experiments was hexane (practical, Eastman Kodak Co. purified with fuming sulfuric acid and with alkaline permanganate; b. p. 62–65°). Visual spectra were observed with a Zeiss Evaluating Grating Spectroscope (light filter BG-7; 2 mm. thick). The quantitative readings were taken in a Beckman photoelectric spectrophotometer.[15] All crystalline substances were dried in high vacuum. Solutions were prepared in a semi-dark room and kept in the dark. Generally, refluxings were carried out for forty-five minutes in an all-glass apparatus, in the dark, in a slow stream of carbon dioxide. In experiments with iodine catalysis, iodine was added in amount 1–2% of the weight of the pigment. The duration of the experiment was thirty to sixty minutes. For the purpose of insolation quartz tubes filled with carbon dioxide were used; the end temperatures never exceeded 26–28°.

The following treatment is recommended for non-crystallizable isomers or for an isomer present only in minor quantities in the chromatograms. After development of the column with commercial petroleum ether (if necessary, containing a suitable percentage of acetone), the solvent is displaced by washing the chromatogram with purest hexane. Each zone is eluted with ice-cold alcohol and sucked through sintered glass into a pre-cooled flask. It is washed alcohol-free by 8–10 shakings with ice-cold water or in an automatic apparatus[16] containing some ice. The solutions are dried rapidly with sodium sulfate, and submitted to immediate optical tests. In order to evaluate the concentrations, a portion of each solution was catalyzed with iodine and the heights of the maxima of the equilibrium mixture thus obtained were used for the calculations. This approximate procedure cannot be used for the estimation of small spectral effects. It can be seen from the curves that the observed iodine equilibrium maxima obtained from some representatives of the same stereochemical set may differ markedly.

We may remark that iodine catalyses should be carried out in light.

γ-Carotene was prepared from *Mimulus longiflorus* flowers and showed, in spite of analytical purity, a considerably depressed melting point (150°, cor.). This phenomenon was discussed recently.[17]

(15) H. H. Cary and A. O. Beckman, *J. Optical Soc. Am.*, **31**, 682 (1941).

(16) A. L. LeRosen, *Ind. Eng. Chem., Anal. Ed.*, **14**, 165 (1942).

(17) L. Zechmeister and W. A. Schroeder, *Arch. Biochem.*, **1**, 231 (1942).

Catalytic Hydrogenation.[18]—5.943 mg. of substance added, in methylcyclohexane and glacial acetic acid, in the presence of 3.04 mg. of PtO_2, 3.26 ml. of hydrogen (22°, 741.5 mm.). 10.138 mg. with 5.47 mg. catalyst added 5.44 ml. of hydrogen (24°, 740.5 mm.). (The second solution retained a trace of color.) $C_{40}H_{56}$. Calcd. 12.0 double bonds. Found: 11.9 and 11.5 double bonds.

Pro-γ-carotene was prepared from *Pyracantha angustifolia*. The analytical constants have been given elsewhere.[19] The molecular extinction coefficients of γ- and pro-γ-carotene are summarized in Table II. Our values for γ-carotene are in reasonable agreement with those estimated from the curve published by Kuhn and Brockmann.[20]

TABLE II

MOLECULAR EXTINCTION COEFFICIENTS OF γ-CAROTENE AND PRO-γ-CAROTENE AND THEIR STEREOISOMERIC EQUILIBRIA AT THE MAXIMA[a] AND MINIMA IN HEXANE

Fresh solution		After heat isomerization		After iodine isomerization	
mμ.	$E^{mol.}_{1\ cm.} \times 10^{-4}$	mμ.	$E^{mol.}_{1\ cm.} \times 10^{-4}$	mμ.	$E^{mol.}_{1\ cm.} \times 10^{-4}$
		γ-Carotene			
493	13.0	*490*	11.4	*486–7*	9.4
478	10.1	478–9	9.6	475	8.6
461–2	14.6	*460*	13.4	*458*	11.4
444	9.6	442	9.3		
436	10.1	*438*	9.4		
		368–70	1.28	369–70	1.72
		349	2.07	*349–50*	2.31
315–20	0.5(0)	310	0.7(2)	310–12	1.02
282	2.95	*283*	2.60	*282–4*	2.57
238–40	0.9(1)	235	1.00	230–40	0.9(8)
		Pro-γ-carotene			
457	11.2			*487–8*	10.9
445–7	10.2			474	9.6
434	11.0			*457*	13.0
304–8	0.6(9)			368–70	1.44
245	2.71			*349–50*	2.30
220–2	1.94			310	0.8(6)
				284	2.52
				235	1.06

[a] The values of the maxima are italicized.

Lycopene was obtained from tomatoes. The samples used were chromatographically homogeneous.

Catalytic Hydrogenation.—11.707 mg. of lycopene with 6.37 mg. of PtO_2 added, in methylcyclohexane and glacial acetic acid, 7.03 ml. of hydrogen (23.5°, 739 mm.). $C_{40}H_{56}$. Calcd. 13.0 double bonds. Found: 12.9 double bonds.

This sample when treated with iodine at 25° reached the photometric end values of the equilibrium mixture within two to three minutes. The extinction heights throughout the spectral curve then remained practically constant (±0.3%) for one to two hours. They were not altered by shaking the fresh solution in air for one to two hours. Such observations show anew that no autoxidation process can be responsible for the *cis*-peak effect.

The molecular extinction coefficients of lycopene and some stereoisomers are given in Table IV. The uncertainties in the highest maxima are respectively: all-*trans*-lycopene, ±6.4%; neo A, ±2%; the "crystallizable isomer,"[1] ±0.5%; prolycopene, ±0.5%; and all-*cis*-lycopene, ±1%. The reason for the large deviation in the case of lycopene is unknown. Data in the literature[21,22] vary for 472–3 mμ between 13.9 and 17.8 × 10⁴. It must also be remarked that the maximum given by Karrer and Rüegger[23] at about 370 mμ was not observed by us in fresh lycopene solutions in hexane and must be due to isomerization.

Neolycopenes A and B.—The main stereoisomers so far obtained from all-*trans*-lycopene are the neo-forms A and B, both adsorbed below the all-*trans* compound on the Tswett column.[24] Their spectral maxima are listed in Table III.

TABLE III

VISUALLY OBSERVED SPECTRAL MAXIMA OF LYCOPENE, NEOLYCOPENE A, AND NEOLYCOPENE B

Solvent	Pigment	Before addition of iodine (mμ)			After addition of iodine (mμ)		
Benzene	Lycopene	521.5	487.5	456.5	518	485	455
Benzene	Neolycopene A	515.5	482	452	518	485	455
Benzene	Neolycopene B	505.5	473.5	443.5	518	485	455
Petroleum	Lycopene	504	473	443	502	471	441.5
ether (b. p. 60–70°)	Neolycopene A	499	467.5	438	502	471	441.5
	Neolycopene B	496	465	430	502	471	441.5

By developing with benzene on lime it is much more difficult to separate the B isomer from A than either of them from the all-*trans* pigment. Nevertheless, the formation of neo B was observed when either lycopene or neo A was kept at 25° or treated with iodine. A solution of neolycopene A which was allowed to stand in the dark at 5° for eight hours showed beside lycopene marked amounts of B when chromatographed. Similarly, neolycopene B yielded neo A and lycopene. The same conversion was observed in sunshine after iodine catalysis.

Prolycopene was isolated from tangerine tomatoes as described earlier.[5] The analytical purity of our samples was confirmed by catalytic hydrogenation: 6.257 mg. substance with 6.46 mg. of PtO_2 added, 3.74 mg. of hydrogen (23°, 745 mm.). 8.950 mg. with 18.14 mg. catalyst added, 5.20 ml. of hydrogen (23°, 745 mm.) $C_{40}H_{56}$. Calcd.: 13.0 double bonds. Found: 13.0 and 12.6 double bonds.

All-*cis*-lycopene.—Several milligrams of prolycopene were melted and kept in a sealed tube in a bath at 115° for five minutes. The tube was plunged into ice-water and the material was dissolved in cold petroleum ether. It was developed on a lime column (20 × 1.8 cm.) with the same solvent containing 5% acetone (the figures on the left denote the width of the chromatographic zones, in mm.)

2 pale pink, heterogeneous (partly lycopene)
2 colorless
25 orange, crystallizable isomer (first band at 476 mμ in petroleum ether)
8 nearly colorless
62 brownish orange: unchanged prolycopene
12 bright yellow: all-*cis*-lycopene

(18) All hydrogenations were carried out in the apparatus designed by A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **12**, 705 (1940).

(19) L. Zechmeister and W. A. Schroeder, *J. Biol. Chem.*, **144**, 315 (1942).

(20) R. Kuhn and H. Brockmann, *Ber.*, **66**, 407 (1933).

(21) A. Smakula, *Angew. Chem.*, **47**, 657 (1934).

(22) E. S. Miller, G. Mackinney and F. P. Zscheile, *Plant Physiol.*, **10**, 375 (1935).

(23) P. Karrer and A. Rüegger, *Helv. Chim. Acta*, **23**, 955 (1940).

(24) In the first papers concerning stereoisomerization neolycopene A was termed "neolycopene" while neolycopene B was observed but not yet identified as a reversible stereoisomer; *cf.* Reference 7; neolycopene B was briefly mentioned by F. W. Went, A. L. LeRosen and L. Zechmeister, *Plant Physiol.*, **17**, 91 (1942).

The bottom layer was eluted with alcohol and treated as described for non-crystallizable isomers.

The composition of the above chromatogram seems to indicate the main steps by which melted prolycopene is converted into its all-*trans* isomer.

TABLE IV

MOLECULAR EXTINCTION COEFFICIENTS OF LYCOPENE AND SOME OF ITS STEREOISOMERS AND OF THEIR STEREOISOMERIC EQUILIBRIA AT THE MAXIMA[a] AND MINIMA IN HEXANE

Fresh solution		After heat isomerization		After iodine isomerization	
mμ	$E^{\text{mol.}}_{1\text{ cm.}} \times 10^{-4}$	mμ	$E^{\text{mol.}}_{1\text{ cm.}} \times 10^{-4}$	mμ	$E^{\text{mol.}}_{1\text{ cm.}} \times 10^{-4}$
			all-*trans*-Lycopene		
503–4	17.2	*502*	14.0	*499*	11.7
489–90	10.0	488	9.7	487	9.2
472–3	18.6	*471*	16.2	*468*	14.1
455	10.8	452	10.3	450	10.0
445	12.3	*444*	11.1	*442*	10.4
372–4	1.22	376–7	1.52	376	1.89
363	1.40	*361–2*	3.5	*362*	3.2
		352	2.41	352	2.30
		347–9	2.48	*347*	2.35
320	0.6(0)	318	0.7(9)	320	1.09
295	5.2	*296*	4.2	*296*	3.9
		264	0.9(5)	266	1.63
		257	1.06	*257*	1.78
240	1.00	237	0.6(0)	244	1.40
226–7	1.92	*228*	1.06	*227*	2.15
			Neolycopene A		
496	10.0			*498–9*	11.9
482	6.9			486	9.2
465	12.2			*467–8*	14.1[b]
446–50	8.0			450	10.0
438–40	9.1			*442*	10.2
379–80	1.89			376	1.53
361	6.8			*361–2*	3.0
350	4.6				
345	4.9				
316	1.42			316	0.8(6)
296–7	2.90			*296*	3.8
268	1.40			264	1.30
255	2.33			*257*	2.06
240–5	1.72			242	1.84
			A crystallizable isomer (Fig. 4, ·· — ··)		
472	9.7				
462	9.3				
445–6	10.2				
314–6	0.7(4)				
297	1.48				
278–90	1.19				
254	2.48				
244	2.02				
233	2.63				
			Prolycopene		
				500	11.8
				486	9.1
				470	14.4
				450	10.0
438	10.3			*444*	10.5
				375	1.55
				361	2.95
				350–3	2.09
				347	2.10
320	0.8(3)			325	0.8(4)
297	1.20			*296*	3.7
285	1.04			265	1.16
				257–8	1.30
233–4	3.8				
			All-*cis*-lycopene		
				498	10.8
				486	8.7
				468	14.1[b]
				452	10.1
432	8.6			*442*	10.7
416–8	7.6				
412	7.8				
				374	1.57
				362	3.1
312–5	1.00			320	1.00
298–302	1.57			*296*	3.8
296	1.54				
253–5	3.3			254	0.9(4)
247–9	3.2			*252*	2.07
				248	1.07
237–8	3.7				
				230	2.76

[a] The values at the maxima are italicized. [b] This value was obtained by iodine catalysis of the all-*trans* form, and was a basis for the calculation of concentrations of the neolycopene A and all-*cis*-lycopene solutions.

Summary

As has already been reported,[4] the bending of a carotenoid molecule by *trans–cis* isomerization causes the appearance of a new spectral maximum (the "*cis*-peak") in a certain ultraviolet region. This phenomenon now has been used for a closer investigation of members of the lycopene stereoisomeric set. No significant contribution to the *cis*-peaks of stereoisomeric mixtures obtained by iodine catalysis, refluxing, etc., is made by all-*trans*-lycopene, all-*cis*-lycopene, or prolycopene, whereas some stereoisomers, especially 6-*cis*-lycopene (neolycopene A), show much higher *cis*-peaks than that of the mixture obtained by iodine catalysis. The situation for the stereoisomeric γ-carotene set is analogous.

Both prolycopene and pro-γ-carotene are observed to undergo stereoisomerization on insolation.

A theoretical interpretation of these observations is proposed. The *cis*-peak results from the absorption of light with transition of the molecule from the normal state to the second excited electronic state. For a molecule with a center of

symmetry (*e. g.*, all *trans*-lycopene or prolycopene) this electronic transition gives rise to no effective dipole moment and hence to no *cis*-peak absorption. Other stereoisomers should show *cis*-peaks of varying intensity, the strongest being shown by the molecule which is *cis* about the central double bond. The *cis*-peak intensity should be roughly proportional to the square of the distance between the center of the conjugated system and the midpoint of the straight line between its ends. By use of such considerations as these some observed stereoisomers of lycopene and γ-carotene are assigned definite spatial structures.

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Conversion of Lutein in a Boric Acid–Naphthalene Melt. I

BY L. ZECHMEISTER AND J. W. SEASE

Although a number of mild reagents or treatments can be used to alter the steric configuration of carotenoids, certain energetic reagents yield crystalline C_{40}-compounds with modified empirical formulas and structure. Since conversions of the latter type involve partial destruction, the conditions must be defined carefully in order to secure reproducible yields. A reaction of this type, the action of cold, concentrated hydriodic acid on carotenes, was described recently.[1]

If crystals of chromatographically homogeneous lutein (xanthophyll, *ex Tagetes*), $HOC_{40}H_{54}OH$, are mixed with naphthalene (in order to depress the melting point), melted in the presence of fused boric acid, and then kept at 140° for a few minutes, a subsequent chromatogram shows many colored layers, none of which contains unaltered lutein. When lutein crystals alone are kept above their melting point for some minutes, stereoisomerization and partial bleaching take place with but negligible amounts of the compounds described in the present paper appearing. The yields are also low in lutein–naphthalene melts but they are substantially increased by the addition to the melt of boric acid or tetraboric acid or boric anhydride. Under the conditions described no analogous conversion of zeaxanthin took place.

The pigments contained in the three main chromatographic layers of the lutein conversion product were crystallized and found to contain one oxygen atom only. Pending a final nomenclature, we designate them, in the sequence of decreasing adsorbability, as desoxyluteins I, II, and III. The yields, based on lutein = 100%, were: 3 to 4% for I, 10% for II, and 3 to 4% for III. The analytical estimations indicate the formula $C_{40}H_{56}O$ ($=H_2$) for all three compounds, which have the following structural features in common: (1) as shown by negative biological assays for vitamin A activity in the rat, an unsubstituted β-ionone ring cannot be present, (2) catalytic hydrogenation indicates eleven double bonds, (3) the oxygen is present in the form of an esterifiable hydroxyl group; when partitioned between methanol and petroleum ether, the com-

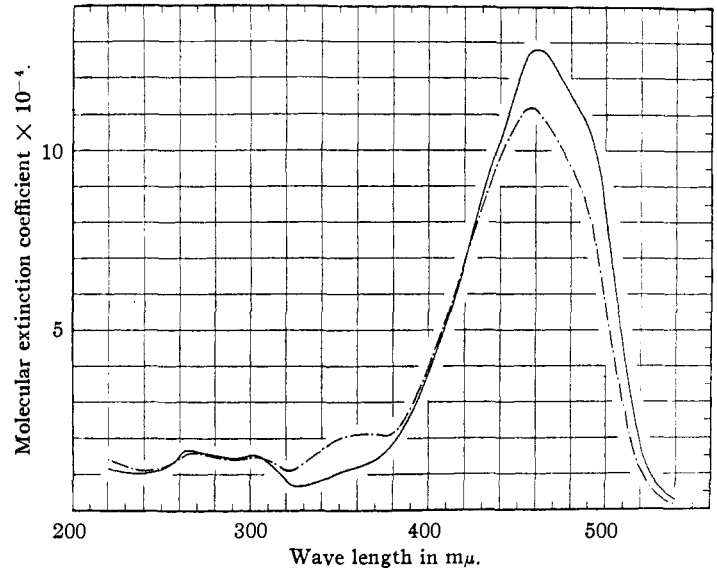

Fig. 1.—Molecular extinction curves of desoxylutein I in hexane: ———, fresh solution of the all-*trans* compound; —·—·—, on iodine catalysis at 25°.

(1) A. Polgár and L. Zechmeister, THIS JOURNAL, **65**, 1528 (1943).