CIS–TRANS ISOMERIZATION AND STEREOCHEMISTRY OF CAROTENOIDS AND DIPHENYLPOLYENES

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I. INTRODUCTION

The literature concerned with the stereochemistry of unsaturated carbon compounds has increased considerably in volume during the last few decades.

However, successful investigations which have demonstrated the presence of a large number of *cis–trans* isomers of a given compound are scarcely known.

Simple and effective methods were needed which would enable the experimenter to differentiate and to purify a multitude of stereoisomers. Such methods can be efficient only if each isomer shows some degree of stability and yet a marked tendency for further isomerization. It is improbable that procedures will be found for the direct synthesis of each stereoisomer. The preparative approach will lie rather in the conversion, under carefully defined conditions, of an easily available or naturally occurring isomer into many others.

In this review we intend to present a survey of some recent progress in the stereochemistry of polyenes.

Compounds which possess a large number of double bonds have been named "polyenes," a term which tacitly implies the aliphatic character and the conjugated position of these bonds. The presence of such a long conjugated system is made evident by the color. The diphenylpolyenes, $C_6H_5(CH{=}CH)_nC_6H_5$, are the simplest synthetic compounds of this type which have been thoroughly studied (see p. 335), but the vegetable and animal kingdoms offer a much greater variety in the form of the carotene-like pigments, the so-called "carotenoids."

A. THE NATURE OF CAROTENOIDS

In this class are found yellow, orange, red, and violet pigments which are free of nitrogen and which may or may not contain oxygen. All carotenoids are built up from isoprene units; the most common ones possess eight such units and, consequently, forty carbon atoms. Those isoprene groups which are located in the central part of the molecule are dehydrogenated thus,

$$\cdots{=}CH{-}\underset{\displaystyle CH_3}{\overset{\displaystyle |}{C}}{=}CH{-}CH{=}\cdots$$

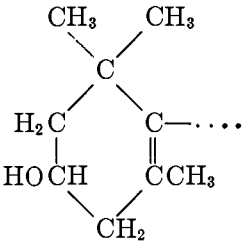
and form part of the chromophore, whereas those near or at the ends of the molecule are hydrogenated.

Carotenoids are insoluble in water but soluble in fats or in fat solvents. A first characterization of a carotenoid pigment can be achieved by the well-known "partition test." If a petroleum ether or benzene solution is shaken with 85 per cent methanol, hydrocarbon pigments are found mainly or entirely in the upper layer ("epiphasic behavior"); in contrast, xanthophylls, i.e., carotenoid alcohols containing at least two free hydroxyl groups, transfer into the lower layer ("hypophasic behavior"). Fully esterified xanthophylls behave like hydrocarbons, whereas monohydroxy carotenoids are epiphasic with respect to 85 per cent methanol but hypophasic (or mainly so) with respect to 95 per cent methanol.

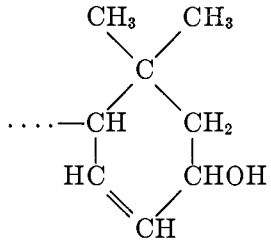
Carotenoids may be hydrocarbons, alcohols, aldehydes, ketones, or acids. α-Carotene, β-carotene, γ-carotene, and lycopene, all of which have the formula $C_{40}H_{56}$, are the most important hydrocarbon carotenoids. The most widespread carotenoid alcohols in nature are the following: cryptoxanthin (a monohydroxy-

β-carotene), $C_{40}H_{55}OH$; zeaxanthin (a dihydroxy-β-carotene), $HOC_{40}H_{54}OH$; and lutein ("xanthophyll", a dihydroxy-α-carotene), $HOC_{40}H_{54}OH$. In some carotenoids, ketone groups occur: for example, in rhodoxanthin, $C_{40}H_{50}O_2$, and capsanthin, $C_{40}H_{58}O_3$. The few carotenoids with acid character are of lower molecular weight and are probably formed by oxidative cleavage of a C_{40}-pigment in the vegetable tissue. Bixin ($CH_3OOCC_{22}H_{26}COOH$), crocetin ($HOOCC_{18}H_{22}COOH$), and azafrin ($(HO)_2C_{26}H_{35}COOH$) belong to this subclass (p. 332).

No carotenoid has yet been found which has an oxygen-containing group in the middle section of the molecule. Hydroxyl or carbonyl groups are usually in a position meta to the quaternary carbon atom in the hydroaromatic ring. The following formulas and those on p. 276 illustrate the main structural features mentioned. For details a monograph may be consulted (102).



Two terminal groups in zeaxanthin; one such group is present in cryptoxanthin



One terminal group in lutein; the other is identical with the formula at the left

B. HISTORY AND METHODS OF *cis–trans* ISOMERIZATION IN THE FIELD OF THE CAROTENOIDS

1. History

So far as is known, the discovery of a second form of bixin by Herzig and Faltis (17) in 1923 was the earliest experimental contribution to the stereochemistry of polyenes. Their observations were interpreted as *cis–trans* rearrangement by Karrer and his associates six years later (21).

In the field of the C_{40}-carotenoids the pioneer observation was made by Gillam and El Ridi (9) in 1935. These investigators reported that if homogeneous β-carotene is washed from an alumina column with petroleum ether (or eluted with methanol) and then re-adsorbed, it separates clearly into two zones which show the spectral bands of β- and α-carotene, respectively. "The lower zone thus contains a pigment formed during the adsorption process, and moreover elutriation and readsorption of either of the two zones gives another separation into two zones with properties as before, on each subsequent adsorption, the process thus being reversible and never complete." The new zone, "pseudo-α-carotene," according to Gillam, cannot be identical with α-carotene because

the latter on similar treatment yields a pigment with maxima at shorter instead of longer wave lengths.

In two subsequent papers (10, 11) in 1936–37 Gillam and his associates made a closer study of the phenomenon and confirmed the complete reversibility of the process

$$\beta\text{-carotene} \rightleftarrows \text{pseudo-}\alpha\text{-carotene}$$

As an explanation of these phenomena, both double-bond migration and geometrical isomerization were discussed by the British authors.

The cause of the isomerization was attributed to the action of the adsorbent, that is, to the chromatographic process itself. This interpretation, however, had to be abandoned. It was observed in 1937 by Cholnoky and the author (104) that capsanthin solutions undergo a spontaneous reversible isomerization; furthermore, nearly simultaneous investigations of lycopene, β-carotene, and cryptoxanthin in collaboration with Tuzson showed that the phenomena are independent of the adsorption process (127, 129). In favorable cases the isomerization can be followed by optical readings without the use of a Tswett column (128).

The reversible changes are spontaneous and occur with much greater rapidity at higher temperatures: for example, when a solution is refluxed. In 1938 Strain (82) reported that heat alters some xanthophyll pigments, and that these then show a complex chromatogram and modified spectral curves. The classic catalyst of *cis–trans* changes, iodine, used previously in the field of bixin and crocetin (p. 322), was introduced into the stereochemistry of C_{40}-carotenoids by Tuzson and the author in 1939 (128). For further historical data *cf.* Jones (18).

Although in our first investigations other processes, e.g., a keto–enol tautomerism in capsanthin, had to be taken into consideration, as the work progressed it was possible to state with increasing certainty (105, 106, 117, 128) that the conversions observed must generally be classified as *cis–trans* rearrangements (*cf.* also the discussions given by Jones (18) and Hunter *et al.* (16)). When the great number of double bonds in a polyene molecule is considered, it becomes evident that a difficult stereochemical problem, which requires special methods, has presented itself.

2. Methods

A long conjugated double-bond system offers many spatial possibilities, but the synthetic as well as the natural polyenes are usually all-*trans* compounds. The following sections of this review will show, however, that this configuration can be easily altered by various thermal, photochemical, or catalytic treatments. Upon such treatment the stereochemical uniformity of the molecules disappears and a complicated mixture of *cis–trans* isomers is formed, in which ordinarily the unchanged portion of the initial all-*trans* pigment still predominates. Theoretically, each possible stereoisomer must be present in such an equilibrium mixture even if only in minute quantity.

The chemical characteristics of the stereoisomers of a given carotenoid are very similar, but fortunately the adsorption affinities are highly dependent on configuration, perhaps more so than any other known physical property. Therefore, the Tswett method of chromatographic adsorption (85, 103) is the only effective technic for the separation and study of the stereoisomers. After suitable development with a solvent, the pigment adsorbed on a vertical column separates into the individual stereoisomers (or groups of them), which appear in distinct zones. Each zone may be cut out, eluted, transferred into another solvent, and examined as desired. The properties, including the readiness, direction, and rate of further *cis–trans* changes, may be studied. Some of the *cis* compounds crystallize easily, while others are labile and can be investigated only in solution.

The chromatographic procedure is also frequently used in order to decide whether or not two pigments from different sources are identical. A mixture of the two compounds is chromatographed: a single zone indicates identity, but two zones point to dissimilarity. This method of the "mixed chromatogram" is very useful in stereochemical work, especially because only minute quantities of material are required for the test.

Spectroscopic methods play an important part in stereochemical studies, as the spectra are strongly influenced by spatial features. The nature of an isomer may be determined rapidly by inspecting the bands in a visual spectroscope before and after the addition of a catalytic amount of iodine, which converts the solute into a stereoisomeric equilibrium mixture. More precise information can be obtained by a study of extinction curves. Stereochemical alteration finds its specific expression not only in the visible region but more particularly in a certain part of the ultraviolet.

It is to be hoped that the combined application of isomerization methods, chromatography, and spectroscopy will stimulate further advance in this field and will finally enable the chemist to deduce the configurations of many *cis–trans* isomers.

After some necessary suggestions concerning nomenclature we shall discuss in more detail the stereochemistry and *cis–trans* isomerization of the C_{40}-carotenoids in Part II, of the carotenoids of lower molecular weight in Part III, and finally, of the diphenylpolyenes in Part IV. In none of these sections has the literature been completely covered, because of present circumstances.

C. NOMENCLATURE

The term "stereoisomeric set" includes all *cis–trans* isomers of a given carotenoid and each stereoisomer is a "member" of the set.

If a pigment is designated by its current name, for example, "β-carotene", this refers usually to the all-*trans* form, in which each double bond possesses *trans* configuration. The prefix "all-*cis*," however, has a more restricted sense. It denotes a compound in which all double bonds which are spatially unhindered in assuming the *cis* configuration are present in the *cis* form. As will be explained later, some double bonds must necessarily remain *trans* because of spatial con-

flicts. Consequently, an "all-*cis*" pigment still retains some *trans* double bonds. Between these limits lies a great number of partially-*cis* isomers for which a special nomenclature is necessary.

We propose the following system (120): Each double bond of the chromophore will be assigned a number which will be italicized in order to avoid confusion with the numbering of carbon atoms: for example, *3,6*-di-*cis*-β-carotene. The lowest number will be given to the double bond in or nearest a β-ionone ring. In the absence of such a cyclic system, an α-ionone ring receives preference over an aliphatic terminal group. Conjugated double bonds in or near such open groups will be assigned the highest numbers. The numbering of entirely aliphatic and unsymmetrical molecules requires special decisions.

Because the configurations of isomers are still unknown in most instances, a temporary nomenclature is in use. It is customary to employ the prefix "neo": for example, neo-β-carotene A. The letters immediately following A in the alphabet designate isomers with weaker adsorbability than A; neo-β-carotene B is adsorbed below neo A. If a pigment (e.g., α- or β-carotene or cryptoxanthin) also yields isomers which adsorb above the all-*trans* form, those with increased affinity are designated as neo T, U, V, etc. The stereoisomers of zeaxanthin, lutein, and capsanthin which are adsorbed above the all-*trans* pigments are designated with the first letters of the alphabet. There is some inconsistency in this nomenclature, which arose on a historical basis.

The use of the prefix "pro" will be discussed later.

II. Carotenoids Containing Forty Carbon Atoms

A. Configuration of Plant Carotenoids in Situ

It can be reliably stated that the overwhelming quantity of the carotenoid pigments present in vegetable tissue possesses an all-*trans* configuration. This follows not only from the results of x-ray investigations of crystals (Hengstenberg and Kuhn (15); Mackinney (49)) and from the interpretation of spectra by Mulliken (56, 57, 58) and by Pauling (62), but also from the chromatographic examination of numerous extracts which have been prepared from various plant organs. The adsorption column usually does not show any appreciable quantity of *cis* compounds in fresh extracts, and even if such zones occur, the possibility of spontaneous isomerization during the extraction and subsequent operations must be excluded before the occurrence of *cis*-compounds in the tissue can be definitely asserted.

The abundant occurrence of the all-*trans* pigments in plants is understandable because they, of all possible spatial structures, possess the lowest energy content and the greatest stability.

The very prevalence of the all-*trans* form in nature stimulated efforts to find exceptions. The exclusive occurrence of a uniform configuration of carotenoids in the vegetable kingdom did not seem probable *a priori*. The first observations in this field, however, were not associated with C_{40}-carotenoids but with the lower-molecular-weight polyenes, bixin and crocetin (p. 322). The simul-

taneous occurrence of partially-*cis*- and all-*trans*-crocetins, which are present as gentiobiosides in the saffron stigma, is remarkable. The *cis*-isomer may easily be converted into the all-*trans* compound by iodine catalysis or irradiation. In discussing these rearrangements with reference to the biosynthesis of carotenoids, Kuhn and Winterstein (39) wrote: "The possibility must be considered that the stigma of saffron will yield substantially more cis-pigment if worked up in the completely fresh state with exclusion of light, heat and catalysts. A photochemical rearrangement of the pigment glucoside perhaps takes place already in the living plant when the flowers open. It will be especially important to test whether other carotene pigments, in particular the precursors of vitamin A, are primarily formed by the plants as labile geometrical isomers of the well-known compounds."

These predictions are in harmony with Schroeder's recent observation (72) that, if stems with the buds of "Monkey flowers" (*Mimulus longiflorus*, Grant) are placed in water, and the flowers allowed to open in diffuse light, substantial amounts of the *cis* compounds, prolycopene and pro-γ-carotene (see below), are present. In contrast, the pigment of the flowers developed on the intact plant in the open contains only the corresponding all-*trans* forms, *viz.*, lycopene and γ-carotene.

It has also been observed that some flowers show a yellow color if there is very little sunshine during their developmental period; during prolonged clear weather, however, the usual orange color appears. Such changes are still open to investigation. Evidently, the configuration of carotenoids *in situ* constitutes a factor which has so far been neglected and which is able to influence both intensity and shade of pigmentation.

That sunshine is necessary for the biosynthesis of some chromophoric structures was demonstrated long ago in the case of chlorophyll. The conjugated double-bond system of carotenoids, however, can be built up in darkness, as evidenced by the formation of pigment in carrots. Sunshine may nevertheless constitute an important factor in directing the initial spatial configuration into its final shape.

The chemical and stereochemical aspects of the carotenoid formation in *Cryptogames* are less well known. For example, van Deventer (4) claimed that light is necessary for the biosynthesis of lycopene and other carotenoids occurring in *Neurospora sitophila*. According to Lederer (43) the pigmentation of a red yeast, *Torula rubra*, is much strengthened by diffuse light. Other cases probably could be found in the literature.

That *cis* structures can be protected and preserved in the vegetable tissue even in prolonged and intense sunshine, is shown by the occurrence of the so-called pro-carotenoids in fruits; the same pigments are stereochemically sensitive when insolated in solutions.

Two representatives of this stereochemically new type of C_{40}-carotenoids, prolycopene, $C_{40}H_{56}$, and pro-γ-carotene, $C_{40}H_{56}$, have recently been found in higher plants. The first qualitative observation was made in collaboration with LeRosen, Went, and Pauling (117), and the isolation of pure crystalline samples

was carried out by LeRosen (45), Schroeder (122, 123), Escue (109), and the author. Whereas the positions of corresponding visible spectral maxima of the bixin and crocetin *cis–trans* pairs differ in wave length by a few millimicrons only, this difference (in petroleum ether) attains 35 mμ and 31 mμ for prolycopene–all-*trans*-lycopene and pro-γ-carotene–all-*trans*-γ-carotene, respectively. As will be shown below, probably six *cis* double bonds are present in prolycopene and five in pro-γ-carotene. These pigments possess characteristically flat extinction curves which undergo spectacular changes upon a photochemical or catalytic treatment.

TABLE 1

Occurrence of prolycopene and pro-γ-carotene in some plants

FAMILY	PLANT	ORGAN	STATE OF DRYNESS	CRYSTALS ISOLATED FROM 1 KG.	REFERENCE
		Prolycopene			
				mg.	
Solanaceae.....	*Lycopersicum esculentum* (Mill.), var. Tangerine tomato	Fruit pulp	Fresh	20	(45)
Palmae.....	*Butia capitata* (Becc.)	Fruit pulp	Fresh		(121)
Palmae.....	*B. eriospatha* (Becc.)	Fruit pulp	Fresh		(121)
Pomoideae.....	*Pyracantha angustifolia* (Schneid.)	Whole fruit	Air-dried	28.4	(123)
Celastraceae.....	*Evonymus fortunei* (L.)	Seeds	Air-dried	11	(109)
Scrophulariaceae..	*Mimulus longiflorus* (Grant)	Petals	Fresh		(124)
		Pro-γ-carotene			
Palmae.....	*Butia capitata* (Becc.)	Fruit pulp	Fresh	0.3	(122)
Palmae.....	*B. eriospatha* (Becc.)	Fruit pulp	Fresh		(122)
Pomoideae.....	*Pyracantha angustifolia* (Schneid.)	Whole fruit	Air-dried	27.7	(123)
Celastraceae.....	*Evonymus fortunei* (L.)	Seeds	Air-dried	0.5	(109)
Scrophulariaceae..	*Mimulus longiflorus* (Grant)	Petals	Fresh		(124)

Representatives of this poly-*cis* class of carotenoids seem to be widespread in nature, as evidenced by the fact that the six plants listed in table 1 belong to five families.

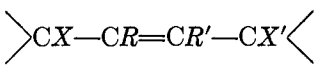
B. THE POSSIBILITY OF STERIC CHANGES IN POLYENIC STRUCTURES

Although the spatial possibilities offered by a long conjugated system are manifold, they have been studied only recently. In 1904 Werner wrote in his textbook (93) that he did not know any compound in which more than two double bonds capable of *cis–trans* changes were present. As late as 1931 Wittig and Wiemer (101) stated that when the number of conjugated double bonds

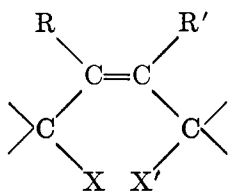
increases, the phenomenon of *cis–trans* isomerism is pushed to the background and finally disappears, because of the increased mobility of the valence electrons (*cf.* 3, 100). Two years later, however, Kuhn (26) correctly summarized the situation by the following statement: ". . . according to the available evidence a strong accumulation of double bonds does not exclude the occurrence of *cis-trans* isomerism. That the higher diphenylpolyenes are known only in one spatial form is due to the inadequacy of the preparative methods." This is in accordance with Ebel's statement (6) that the non-existence of many isomers foreseen by theory is caused by their rapid rates of rearrangement.

It can be reliably stated on the basis of recent experimental evidence, that the length of an aliphatic double-bond system does not affect its ability to assume various *cis–trans* configurations; in some carotenoid sets a dozen stereoisomers have been observed.

We shall now proceed to a discussion of the number of theoretically possible *cis–trans* isomers of a given conjugated system. First of all, it must be stressed that not all double bonds of the system are necessarily available for a spatial rearrangement. They can be divided into "stereochemically effective" and "stereochemically ineffective" double bonds, the latter being hindered by some spatial conflict in assuming *cis* configuration. In a C_{40}-carotenoid, the chromophore of which is composed of dehydrogenated isoprene groups, only one double bond in each C_5 unit of the aliphatic chain is able to assume *cis* configuration. According to Pauling (62), these are those double bonds which carry methyl side chains, and in addition the central double bond; the latter has a privileged structural and stereochemical position (see the formulas on page 276). The stereochemically ineffective conjugated double bonds are those which are located in a ring or which are adjacent to a C—CH_3 group. The latter type of double bond is ineffective because of the steric interaction between the hydrogen of CH and the methyl of C—CH_3. Thus, in a chain with the structure



the *cis* form will be oriented in the following way:



If X and X′ are both hydrogen atoms, they will be 1.7 Å. apart. This is somewhat less than the usual distance of van der Waals contact, but the strain can be removed in large measure by small rotations out of coplanarity. If, however, X = methyl (or some similar group) and X′ = H, the distance is only 1.6 Å. (figure 1), i.e., only half the distance of the van der Waals contact. The *cis* form would, therefore, certainly be unstable. Hence Pauling (62) concluded that in

the open chain of carotenoids, *cis* configuration will be assumed only by those double bonds which are of the type $\rangle CH—CR{=}CR'—CH\langle$, that is, which are adjoined by two CH groups.

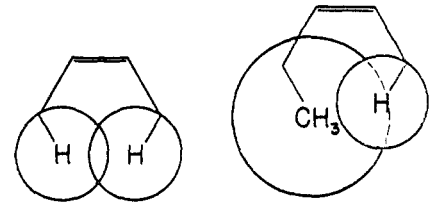
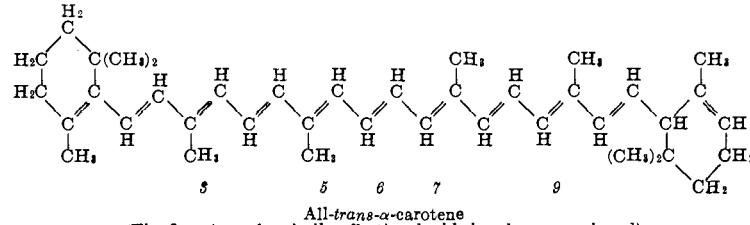
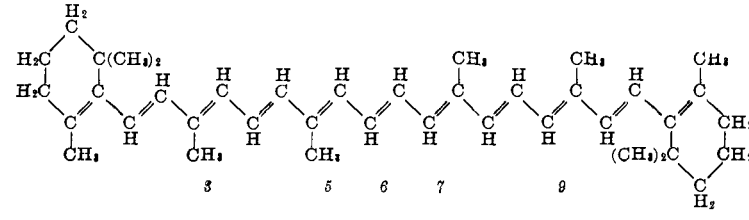


FIG. 1. Overlapping of hydrogen atoms in —CH—CR=CR—CH— and of hydrogen and methyl in —CH—CR=CR—CCH_3 with *cis* configuration.

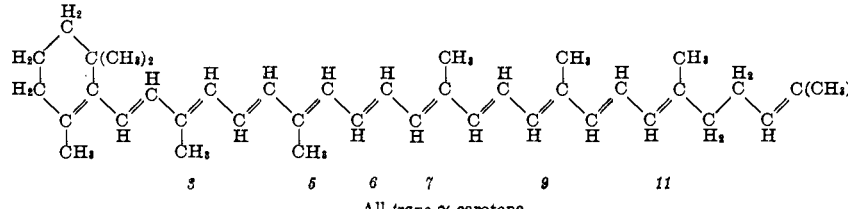
On the basis of these considerations the number of possible *cis–trans* isomers is much less in the highly branched carotenoid molecule than in an unbranched aliphatic compound with an equal number of conjugated double bonds. The positions of the stereochemically effective double bonds in the four important natural polyene hydrocarbons, the carotenes and lycopene, are indicated in the formulas below (the stereochemically effective double bonds are numbered):



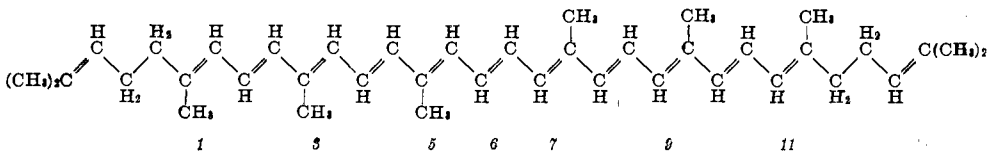
All-*trans*-α-carotene
(The five stereochemically effective double bonds are numbered)



All-*trans*-β-carotene
(The five stereochemically effective double bonds are numbered)



All-*trans*-γ-carotene
(The six stereochemically effective double bonds are numbered)



All-*trans*-lycopene
(The seven stereochemically effective double bonds are numbered)

In order to calculate the number of possible stereoisomers (117), the carotenoids (and related polyenes) are divided into two subclasses containing "unsymmetrical" and "symmetrical" chains, respectively. In the first type the two halves of the molecule are dissimilar; in the second type they are identical.

For unsymmetrical chains with n stereochemically effective double bonds, the number of stereoisomers $N = 2^n$. For symmetrical chains with n odd, the corresponding value is

$$N = 2^{(n-1)/2} \cdot (2^{(n-1)/2} + 1)$$

whereas with n even it is

$$N = 2^{n/2-1} \cdot (2^{n/2} + 1)$$

TABLE 2

Numbers of cis–trans isomers for unsymmetrical and symmetrical chains containing n stereochemically effective double bonds

UNSYMMETRICAL CHAINS		SYMMETRICAL CHAINS	
n	Number of isomers	n	Number of isomers
1	2	1	2
2	4	2	3
3	8	3	6
4	16	4	10
5	32	5	20
6	64	6	36
7	128	7	72
8	256	8	136
9	512	9	272
10	1024	10	528
11	2048	11	1056
12	4096	12	2080

Table 2 shows that with increasing n the values for N increase rapidly. However, in natural carotenoids known at the present time the number of stereochemically effective double bonds is only four to seven, and the calculated number of stereoisomers varies between 10 and 128 (table 3) (117). Figure 2 gives the skeleton models for all twenty members of the β-carotene set, and figure 3 the all-*trans*, mono-*cis*, and di-*cis* members of the α-carotene set.

C. METHODS OF *cis–trans* ISOMERIZATION

The configuration of all-*trans* carotenoids is stable in the crystalline state. Numerous methods are available, however,—some as yet almost unexplored,—

which can be utilized for the partial stereoisomerization of an all-*trans* compound or any of its isomers. Although the existence of such spatial changes may be

TABLE 3

Calculated number of stereoisomers for naturally occurring carotenoids and for some of their conversion products

PIGMENT	SYMMETRICAL (*s*) OR UNSYMMETRICAL (*u*) MOLECULE	NUMBER OF STEREOCHEMICALLY EFFECTIVE DOUBLE BONDS	CALCULATED NUMBER OF STEREOISOMERS
α-Carotene	*u*	5	32
β-Carotene	*s*	5	20
γ-Carotene	*u*	6	64
Lycopene	*s*	7	72
Cryptoxanthin	*u*	5	32
Rubixanthin	*u*	6	64
Gazaniaxanthin	*u*	6	64
Lycoxanthin	*u*	7	128
Lutein	*u*	5	32
Zeaxanthin	*s*	5	20
Physalien	*s*	5	20
Lycophyll	*s*	7	72
Rhodoviolascin	*u*	7	128
Rhodoxanthin	*s*	5	20
Capsanthin	*u*	5	32
Capsorubin	*s*	5	20
Astacin	*s*	5	20
Astaxanthin	*s*	5	20
Semi-β-carotenone	*u*	5	32
β-Carotenone	*s*	5	20
α-Citraurin	*u*	5	32
β-Citraurin	*u*	5	32
Methylbixin	*s*	5	20
Bixin	*u*	5	32
Norbixin	*s*	5	20
Crocetin	*s*	5	20
Methylcrocetin	*u*	5	32
Dimethylcrocetin	*s*	5	20
Azafrin	*u*	4	10
Vitamin A	*u*	2	4

demonstrated by optical methods (for example, spectroscopy, colorimetry, polarimetry, etc.), chromatographic analysis is the only means available for the separation of the individual isomers.

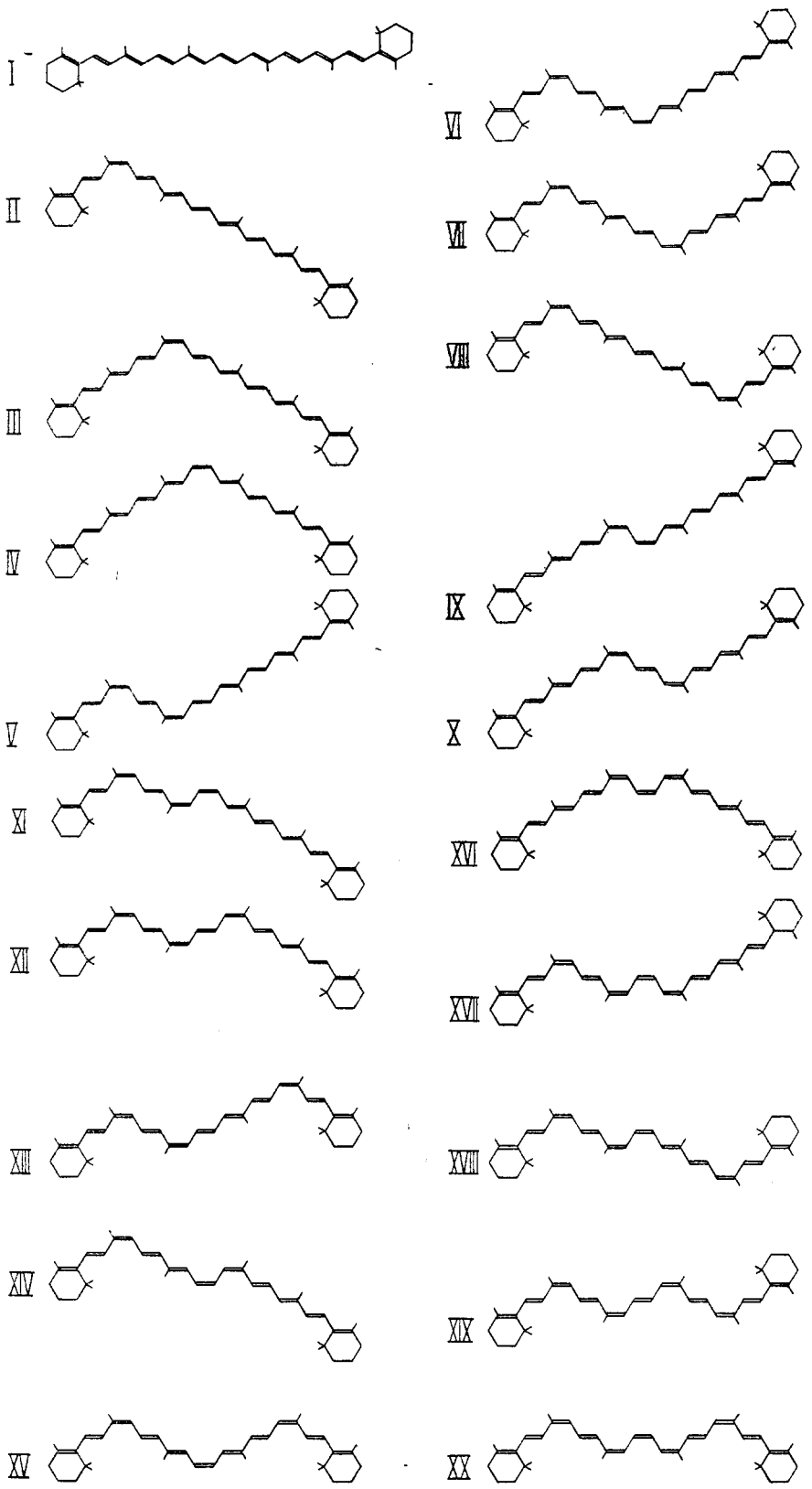


FIG. 2. Skeleton models of the twenty possible stereoisomers of β-carotene: all-*trans*-β-carotene, three mono-*cis*-β-carotenes, six di-*cis*-β-carotenes, six tri-*cis*-β-carotenes, three tetra-*cis*-β-carotenes, and all-*cis*-β-carotene.

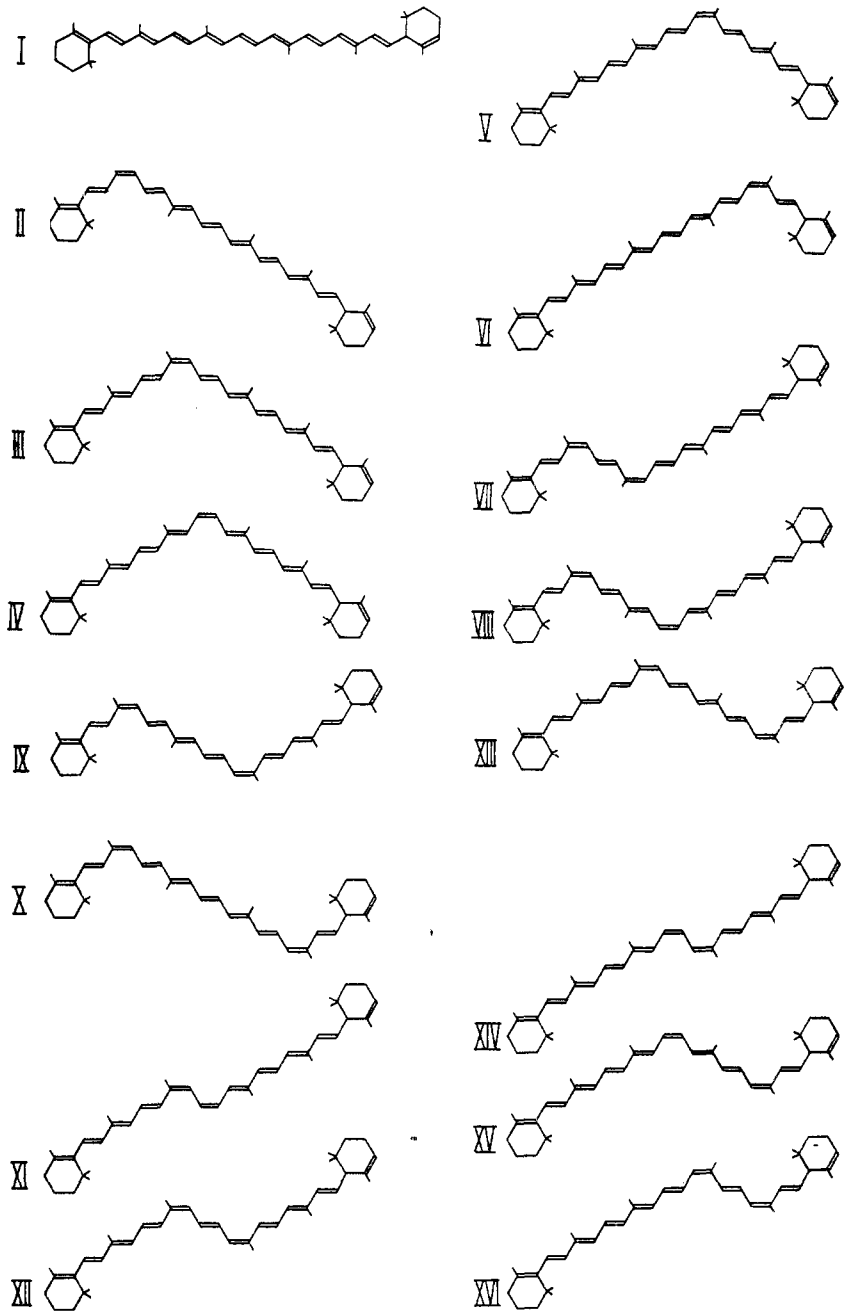


FIG. 3. Skeleton models of sixteen of the possible thirty-two stereoisomers of α-carotene: all-*trans*-α-carotene, five mono-*cis*-α-carotenes, and ten di-*cis*-α-carotenes.

No pigment zone observed on the Tswett column after an isomerization experiment should, however, be accepted without further criticism as containing

a member of the initial stereochemical set, because the possibility of a slight destruction or oxidation by air exists. Such processes can even become predominant under drastic conditions. Products of moderate oxidation are adsorbed near the top of the column, whereas cleavage products possessing a considerably shortened chromophore may pass into the chromatographic filtrate, to which they impart a pale color or a fluorescence when illuminated with ultraviolet light. In most cases the product of these unwelcome processes can easily be differentiated from stereoisomerized pigments: the isomerization is reversible, in contrast to oxidation and cleavage. When any stereoisomer is submitted to the treatment by which it was formed (or to an equivalent treatment), members of the same stereochemical set must appear.

This isomerization can be rapidly achieved by iodine catalysis. Because the all-*trans* isomer thus formed is present in preponderant or at least substantial amount, it can easily be separated on a new column and compared in a mixed chromatogram with an all-*trans* sample from another source. Pigment zones formed by current methods of isomerization which do not respond to a reversibility test represent losses in stereochemical work.

The following methods will now be considered somewhat more in detail: (*1*) thermal isomerization in solution; (*2*) thermal isomerization achieved by melting crystals; (*3*) isomerization induced by iodine catalysis or (*4*) by acid catalysis; and finally (*5*) photo-isomerization.

1. Thermal cis–trans isomerization in solution

To this category belong spontaneous isomerization at room temperature and isomerization on heating or refluxing in the absence of light or catalysts.

The phenomenon of spontaneous isomerization, which starts immediately on solution of crystals and proceeds much more rapidly upon refluxing, was observed with lycopene, β-carotene, and cryptoxanthin in collaboration with Tuzson (129). The changes which lutein or zeaxanthin solutions undergo on heating were described by Strain (81, 82, 83) and also by Tuzson (128) and Cholnoky, Polgár, and the author (106, 107). While several of Strain's observations were the first in this field, the reversibility of the process was not claimed in this early work nor was the action of heat clearly differentiated from an alleged effect of the column which had been advanced by Gillam and El Ridi (10).

The spontaneous isomerization of all-*trans* carotenoids at room temperature is a slow process; its rate depends on the solvent and on the pigment structure. We found, for example, that the following percentages of the starting material had isomerized in benzene or petroleum ether solution within a day: α-carotene, β-carotene, cryptoxanthin, and capsanthin, 1 to 2 per cent; gazaniaxanthin and zeaxanthin, 4 to 5 per cent; capsorubin, 8 per cent; lycopene, 10 per cent. According to Carter and Gillam (2), if β-carotene solutions are stored at −2°C. in the dark and protected from air, less than 3 to 4 per cent will undergo stereoisomerization in three months. Even partially-*cis* isomers may be preserved under similar conditions; thus, certain β-carotene isomers showed only 5 to 6 per cent re-isomerization after two months. The influence of the temperature on the stability of dissolved all-*trans*-β-carotene is illustrated in table 4.

When a dilute benzene or petroleum ether (b.p. 60–80°C.) solution of an all-*trans* carotenoid is refluxed, the equilibrium (or steady-state) mixture is reached within 15 to 60 min. The extent of this isomerization for certain carotenoids is summarized in table 5. The behavior of partially-*cis* compounds may vary within broad limits. Many such isomers have conversion rates which are similar to those of the all-*trans* form. Prolycopene and pro-γ-carotene are, however, so thermostable that their extinction curves remain practically unaltered when the solutions in petroleum ether are refluxed for 30 min. (116).

Comparative data for five members of the β-carotene set (65) are given in table 6.

The rate of isomerization of β-carotene is higher in the non-polar solvent toluene, than in the polar solvent nitromethane (16).

TABLE 4

Percentage of β-carotene stereoisomers with decreased adsorption affinity formed at various temperatures in petroleum ether–benzene solution (Carter and Gillam (2))

TEMPERATURE	DURATION OF THE EXPERIMENT	ISOMERIZED FRACTION
°C.		*per cent*
20	1 day	1
	7 days	5.5
	49 days	11.1
40	1 hr.	4.0
	3 hr.	5.4
	24 hr.	11.2
60	1 hr.	7.5
	3 hr.	9.7
80	1 hr.	8.5
	3 hr.	31.9
	24 hr.	34.1

2. Cis–trans isomerization by melting crystals

If crystals of a carotenoid are kept a few degrees above their melting point, both reversible and irreversible changes occur. The conversion becomes manifest not only by a loss in the initial color intensity, which may decrease by more than half, but also by the appearance of fluorescing material in the filtrate of a chromatogram. The extent of cleavage is less if the pigment possesses a low melting point, if the duration of the experiment is only 1–5 min., and if the fusion temperature is depressed by addition of naphthalene. In no case do true equilibrium mixtures seem to be formed.

In practice the pigment is melted in a sealed tube filled with carbon dioxide. Then the melt is solidified by rapid cooling, dissolved, and chromatographed. Whether or not a zone contains a member of the initial set can be established by spectroscopy, by the partition test, or by mixed chromatograms.

The data in table 7 show that the all-*trans* form is by no means present in greatest quantity in the stereoisomeric mixture obtained, the composition of

TABLE 5

Cis-trans isomerization of all-trans carotenoids caused by refluxing solutions

PIGMENT	SOLVENT	DURATION OF REFLUXING	RATIO OF STEREOISOMERS (PER CENT OF PIGMENT RECOVERED)	REFERENCES
		minutes		
α-Carotene	Petroleum ether (b.p. 60–70°C.)	30	All-*trans*:neo U:neo B = 92:4:4	(120)
β-Carotene	Petroleum ether (b.p. 60–70°C.)	60	All-*trans*:neo U:neo B:neo E:labile isomer = 86:4:8:1:1	(65)
Lycopene	Petroleum ether (b.p. 70–80°C.)	30	All-*trans*:neo forms = 45:55	(129)
Cryptoxanthin	Ligroin (b.p. 120°C.)	60	All-*trans*:neo A:neo B = 62:32:6	(113)
Gazaniaxanthin	Benzene	30	All-*trans*:neo forms = 70:30	(125)
Lutein	Benzene	30	All-*trans*:neo forms = 89:11	(128)
Zeaxanthin	Benzene	30	All-*trans*:neo A:neo B = 70:24:6	(106)
Physalien	Petroleum ether (b.p. 70–80°C.)	60	All-*trans*:neo forms = 58:42	(106)
Taraxanthin	Benzene	30	All-*trans*:neo forms = 85:15	(128)
Capsanthin	Benzene	30	All-*trans*:neo forms = 80:20	(105)
Capsanthin dipalmitate	Petroleum ether (b.p. 70–80°C.)	30	All-*trans*:neo forms = 64:36	(105)
Capsorubin	Benzene	30	All-*trans*:neo forms = 80:20	(105)

TABLE 6

Relative colorimetric values of β-carotene and some of its stereoisomers formed by refluxing for 60 min. in petroleum ether (b.p. 60–70°C.)

STARTING MATERIAL	RELATIVE PHOTOMETRIC VALUES (PER CENT)				
	Neo U	All-*trans*	Neo B	Neo E	Labile isomer
Neo-β-carotene U	31	40	19	10	
All-*trans*-β-carotene	4	86	8	1	1
Neo-β-carotene B	4	50	40	3	3
Neo-β-carotene E	14	49	19	18	
Labile isomer	10	22	24	30	14

which is, of course, dependent on the conditions of the experiment. Some partially-*cis* pigments are only slightly less stable in the melt than the all-*trans*

form; for example, after having been kept in the melt at 115°C. for 5 min., more than half of prolycopene was unchanged. The isomerized portion contained partially-*cis* members of the set and some all-*cis*-lycopene but only a trace of the all-*trans* form (45).

3. Cis–trans isomerization by iodine catalysis at room temperature

It is well known that iodine is a powerful catalyst which strongly influences the spatial structure of ethylene derivatives. In the field of C_{40}-carotenoids

TABLE 7

Stereoisomers formed by melting crystals of carotenoids

STARTING MATERIAL	TEMPERATURE	DURATION OF FUSION	RATIO OF STEREOISOMERS	REFERENCES
	°C.	*minutes*		
All-*trans*-α-carotene	195–200	15	All-*trans*:neo U:neo V:neo W:neo X:neo B:neo D = 35:12:6:19:7:14:7	(120)
Neo-α-carotene U	74	15	All-*trans* (with A):neo U:neo X:neo B = 23.5:66:7:3.5	(120)
All-*trans*-β-carotene	190	15	All-*trans*:neo U:neo V:neo A:neo B:neo E:labile isomer = 33:19:4:8:24:8:4	(65)
Neo-β-carotene U	135	15	All-*trans*:neo U:seven minor isomers = 22:40:38	(65)
All-*trans*-cryptoxanthin	170	2	Some neo A, no neo U, mostly unchanged all-*trans*	(113)
	170	10	All-*trans*:neo U:neo A = 48:22:30	(113)
	170	15	All-*trans*:neo U:neo A:neo B = 49:7:38:6	(113)
All-*trans*-cryptoxanthin (with naphthalene)	115	5	Mostly unchanged all-*trans*, some neo A, less neo B, no neo U	(113)
All-*trans*-zeaxanthin (with naphthalene)	160	15	All-*trans*:neo A:neo B:neo C = 56:17:17:10	(113)
All-*trans*-capsanthin	180	1	All-*trans*:neo forms A + B + C = 29:71	(67)

it was first used in collaboration with Tuzson (128) when lycopene, β-carotene, lutein, cryptoxanthin, zeaxanthin, and taraxanthin were investigated. The earliest detailed studies referred to zeaxanthin and its ester physalien (with Cholnoky and Polgár (106)), and to capsanthin, capsorubin, and their palmitates (with Cholnoky (105)).

If iodine is added to a solution of an all-*trans* carotenoid, in quantities of 1 to 2 per cent of the pigment, rapid steric changes take place at room temperature. This leads, in most cases, to an equilibrium mixture in which all members of the

stereoisomeric set should theoretically be present. In practice, however, subsequent chromatography shows that only a limited number of stereoisomers, two to twelve, can be detected on the usual scale of laboratory work. Those members of the set which contain many *cis* bonds and which, therefore, are spectroscopically very different from the all-*trans* form, are not formed in detectable quantities.

The composition of the iodine equilibrium mixture is usually different from that of the equilibrium mixture obtained by the refluxing of solutions. This difference lies not only in the ratio in which the members of the set are present but even in the presence or absence of detectable amounts of certain isomers. For example, only a little neolutein B is obtained when a solution of all-*trans*-lutein is refluxed, but this isomer appears in substantial quantities upon iodine catalysis in the cold (128). The method of isomerization also influences the ratio of neozeaxanthins A and B and of neocapsanthins A and B (105). A limiting case is given by prolycopene and pro-γ-carotene, which are only slightly isomerized by refluxing but in contrast are extremely sensitive to iodine. When they are treated with this catalyst, no detectable amounts of the starting material are present in the equilibrium mixture.

The outstanding feature of the stereochemical rearrangement which takes place in the presence of iodine is the reversibility of the process. If an isomer is separated on a Tswett column, eluted, transferred into a suitable solvent, and catalyzed again, all the pigments observed in the first chromatogram appear in a subsequent chromatogram. Theory requires that each member of a set give the same equilibrium mixture.

If the extinction values for such a mixture have been established by adding the catalyst to a solution of a weighed quantity of a crystalline member of the set, the concentration of any isomer belonging to the set, whether crystallizable or not, can be rapidly established. Some factors to be discussed later, however, may make such estimations inaccurate to the extent of $\pm$ 0.5 to 5 per cent.

In order to obtain information on the rates of isomerization, spectrophotometric readings can be made at regular intervals of time at a definite wave length until constancy (or quasi-constancy) has been reached. A sample of the solution may then be chromatographed and the ratio of unchanged to isomerized pigment may be determined.

Exact measurement of the kinetics of iodine catalysis is not yet available. The time needed to achieve steric equilibrium evidently is dependent on the concentrations and temperature. For practical purposes it can be stated that if the concentration of the pigment is of the order of magnitude of 0.1 mg. per milliliter of petroleum ether or benzene (1/5000 molar), and if the weight of the iodine is 1 to 3 per cent of the pigment, then at 25°C. equilibrium is reached within 15 to 60 min. (in many cases within a few minutes).

The dependence of the rate of isomerization on the quantity of catalyst is illustrated in table 8 (45).

As in many other cases of iodine catalysis, the stereoisomerization of carotenoids is promoted by light. One can even claim that for all practical purposes daylight or illumination with a lamp is necessary, although over-exposure may

cause destruction. Individual carotenoids behave very differently; lycopene is exceptionally sensitive. However, the behavior of lycopene will be considered and illustrated later in the discussion of the so-called "*cis*-peak" effect (p. 302).

Iodine is not only a catalyst for stereoisomerization but may also cause the formation of faintly colored cleavage products. This side reaction is shown through losses in the total color intensity which are established by a photometric balance after catalysis, chromatography, and recovery. In many cases this loss is negligible, but with prolycopene (45) it amounted to 30 per cent in some experiments, although it was much less in others. In contrast, 97 per cent of the initial pigment was recovered in a blank determination in which no iodine was added.

Strain (84) observed that iodine catalysis of zeaxanthin produced not only the well-known stereoisomers, but also some pigments which were usually formed by acid catalysis. If the iodine solution was mixed with a little pyridine, quinoline, or dimethylaniline, the by-products did not appear. This result would possibly indicate the formation of traces of acid under the influence of iodine.

TABLE 8

Influence of the quantity of iodine on the rate of stereoisomerization of prolycopene (45)

RATIO OF GRAM-ATOMS OF IODINE TO GRAM-MOLECULES OF PIGMENT	AMOUNT OF IODINE (PER CENT OF THE PIGMENT)	EXTENT OF ISOMERIZATION AT 25°C. WITHIN 1 MIN. (PER CENT OF THE PIGMENT RECOVERED)
1:20000	0.0013	2
1:2000	0.013	37
1:200	0.13	93

In spite of these side reactions, which were rather emphasized above, iodine catalysis in general is a powerful and reliable tool in research dealing with the stereochemistry of polyenes. We shall give now some specific results.

(*a*) *Hydrocarbon carotenoids:* The composition of the equilibrium mixture of pigments formed reversibly by iodine catalysis from α-carotene, β-carotene, 5,6-dihydro-α-carotene, and 5,6-dihydro-β-carotene is summarized in table 9 (65, 66, 120).

For lycopene the following data are available (128):

Minutes	0	5	30	90	180
Ratio of all-*trans* to neo forms	100:0	82:18	71:29	57:43	53:47

A mixture of neolycopenes A and B behaved as follows:

Minutes	0	5	30	90
Ratio of all-*trans* to neo forms	0:100	13:87	19:81	30:70

(*b*) *Monohydroxy-carotenoids:* The behavior of some members of the cryptoxanthin set (113) is given in table 10. Gazaniaxanthin, a monohydroxy-γ-

TABLE 9

Relative colorimetric values of some members of hydrocarbon sets as formed by iodine catalysis in petroleum ether solution in daylight

Duration: 30 min.; for β-carotene, 60 min.

STEREOISOMERIC SET	STARTING MATERIAL	RELATIVE COLORIMETRIC VALUES (IN PER CENT OF PIGMENT RECOVERED)					
		Neo U	Neo V	Neo W	All-*trans*	Neo B	Neo forms C + D + E
α-Carotene	Neo U	11	3.5	21	49.5	12	3
	Neo V	11.5	4	19.5	50	13	2
	Neo W	15.5	3.5	18.5	43.5	17	2
	All-*trans*	14.5	3	15.5	51.5	13	2.5
	Neo B	10.5	3	15	57	12.5	2
	Neo forms C + D + E	10	2.5	21	51.5	9.5	5.5
		Neo U	All-*trans*	Neo B	Neo E	Labile isomer	
β-Carotene	Neo U	24	47	24	3	2	
	All-*trans*	22	48	25	3	2	
	Neo B	21	51	23	3	2	
	Neo E	20	48	24	4	4	
	Labile isomer	18	45	16	13	8	
		Neo T	Neo U	All-*trans*	Neo A	Neo B	
5,6-Dihydro-α-carotene	Neo T	23	15	54	8		
	Neo U	5	22	55	18		
	All-*trans*	4	20	56	16	4	
	Neo A	2	17	49	26	6	
	Neo B	1	19	64	11	5	
		Neo forms U + V	All-*trans*		Neo forms A + B + C		
5,6-Dihydro-β-carotene	Neo U	17	63		20		
	Neo V	17	69		14		
	All-*trans*	18	67		15		
	Neo A	21	64		15		
	Neo forms B + C	16	70		14		

TABLE 10

Relative photometric values of some members of the cryptoxanthin set as formed by iodine catalysis in petroleum ether solution in 60 min. at room temperature in daylight

STARTING MATERIAL	RELATIVE COLORIMETRIC VALUES (PER CENT OF PIGMENT RECOVERED)			
	Neo U	All-*trans*	Neo A	Neo B
Neo U	23	55	22	
All-*trans*	18	59	18	5
Neo A	20	57	23	
Neo B	21	55	17	7

carotene (71, 125), attained equilibrium within 15 min. under the conditions employed, and this time was also sufficient if iodine was added to the mixture of the isomers. The following figures refer to the catalysis of the all-*trans* form; the stereoisomers, the chromatographic separation of which is difficult, are given as Groups I and II.

Minutes of catalysis	5	15	30
Ratio of all-*trans:* Group I: Group II	72:20:8	56:29:15	56:29:15

(*c*) *Xanthophylls with two or more hydroxyl groups:* The behavior of lutein (128) and of zeaxanthin (106) is presented in tables 11 and 12. For taraxanthin

TABLE 11

Relative photometric values of some members of the lutein set formed by iodine catalysis in petroleum ether in 30 min. in daylight

STARTING MATERIAL	RELATIVE PHOTOMETRIC VALUES (PER CENT OF PIGMENT RECOVERED)		
	Neo A	Neo B	All-*trans*
Neo A	20	22	58
Neo B	20	24	56
All-*trans*	17	23	60

TABLE 12

Relative photometric values of some members of the zeaxanthin set formed by iodine catalysis in benzene in 30 min. in daylight

STARTING MATERIAL	RELATIVE PHOTOMETRIC VALUES (PER CENT OF PIGMENT RECOVERED)			
	Neo A	Neo B	Neo C	All-*trans*
Neo A	30	15	3	52
Neo B	10	19	3	68
Neo C	12	18	3	67
All-*trans*	10	21	3	66

only a few data are available (128). The esterification of the hydroxyls of xanthophylls leaves the iodine sensitivity essentially unaltered.

(*d*) *Ketones:* The behavior of all-*trans*-capsanthin in benzene solution is illustrated by the following experiment (105), which was carried out at 20°C.:

Minutes of catalysis	1	15	900
Ratio of all-*trans:* neo A: neo B: neo C	78:8:11:3	69:15:11:5	66:23:7:4

The behavior of the neo forms is similar.

The following figures for capsanthin dipalmitate are typical; the same equilibrium was reached rapidly from both sides and remained constant over a long period of time.

STARTING MATERIAL	RATIO OF ALL-*trans* TO NEO FORMS			
	After 15 min.	After 30 min.	After 60 min.	After 24 hr.
All-*trans*	64:36	64:36	67:33	68:32
Neo forms A + B	64:36	66:34	66:34	66:34

Capsorubin in benzene isomerized in the following manner (105):

STARTING MATERIAL	RATIO OF ALL-*trans* TO NEO FORMS		
	After 15 min.	After 60 min.	After 15 hr.
All-*trans*	72:28	75:25	68:32
Neo forms	67:33	65:35	

Capsorubin dipalmitate in benzene after a 15-min. catalysis had isomerized to the extent of 26 per cent and did not isomerize further on standing for a day at 20°C.

4. Cis–trans isomerization by acid catalysis

The quality and quantity of the products which are formed by the action of acids on carotenoids depend upon the conditions, especially upon the strength and the concentration of the acid. In the extreme case total destruction and bleaching occurs, but under moderate conditions structural changes may take place which cannot be qualified as cleavage. To this category belong the reduction of carotenes with cold commercial concentrated hydriodic acid (66) and the formation of "desoxyluteins" in the boric acid melt, investigated by Sease and the author (126). Under still milder conditions which are, however, by no means equal for each carotenoid, spatial rearrangements chiefly take place.

The greater acid sensitivity of the xanthophylls is a well-known characteristic. Some xanthophylls or hydroxyketones (violaxanthin, fucoxanthin, capsanthin, capsorubin, etc.) give dark blue colorations with strong hydrochloric acid.

According to Kuhn, Winterstein, and Lederer (41) lutein is very sensitive toward small amounts of organic acids when solutions in methanol are refluxed; the rotatory power increases while the melting point decreases. These observations were confirmed and extended by Strain (82, 84). Simultaneously, an investigation by Quackenbush, Steenbock, and Peterson (69) showed the formation of several new polyene pigments from the xanthophylls of forage and from pure lutein under the influence of different acids. It is unknown, at present, which, if any, of these pigments were formed by steric changes. Some further observations have recently been made by Strain (84) on several xanthophylls, and by Strain and Manning (86) on fucoxanthin.

Acid isomerizations of carotenoids can be prevented by the addition of such organic bases as pyridine, quinoline, or dimethylaniline (Strain (84)).

In the author's laboratory, the acid catalysis of a polyene is usually carried out by shaking a petroleum ether or benzene solution with more or less con-

centrated aqueous acid under carbon dioxide, for example, for half an hour. The catalytic process is accelerated in this technique by the incessantly changing interface.

When β-carotene (66) was treated with commercial concentrated hydrochloric acid, it was converted into irreversible pigments to a slight extent only, and the composition of the stereoisomeric mixture recovered was as follows: all-*trans:* neo U:neo B:neo E:unnamed labile isomer = 50:23:23:3:1. The action of 28 per cent hydriodic acid upon α-carotene gave this mixture: all-*trans*: neo U:neo V:neo W:neo B:neo C = 49:13:2:15:8:9:4. The members of both sets were destroyed by the action of 56 per cent hydriodic acid. The acid isomerization behavior of 5,6-dihydro-α-and-β-carotenes prepared by Polgár and the author (66) was similar. Pro-γ-carotene (122), after interaction with concentrated hydrochloric acid for 30 min., yielded a stereoisomeric mixture of the γ-carotene set in which unchanged starting material was absent; some irreversible products were formed.

5. Photochemical cis–trans isomerization

All carotenoid solutions which have been investigated up to the present time are photosensitive, but the degree to which they possess this characteristic shows considerable variation. It is dependent both on the chemical and on the spatial structure. Visible light is much more effective than wave lengths in the ultraviolet region. Most of the experiments have been conducted by exposing the pigment solutions to intense sunshine ("insolation") in transparent quartz tubes from which the air had been displaced with carbon dioxide.

In order to differentiate the photo-isomerization from the thermal process, the temperature should be noted and, if necessary, a cooling device should be employed.

Of course, *cis–trans* isomerization is only one of the processes which take place. It competes with the more or less rapid destruction of pigment which is probably promoted by traces of oxygen and by accidental catalysts. We have observed complete bleaching in some cases. Thus a sharp differentiation between cleavage and *cis–trans* rearrangement is necessary and may be achieved by chromatographing the irradiated solution and subsequently testing the reversibility of the pigment in each zone. The partition behavior should also be checked.

If an all-*trans* carotenoid is exposed to light, both *trans* → *cis* isomerization and destruction will cause a decrease in the color intensity. When a pigment which contains only one or two *cis* double bonds is irradiated, the experimental conditions will determine whether the color-decreasing effect of destruction or the color-increasing effect of *cis* → *trans* isomerization will prevail. However, if a poly-*cis* compound is exposed to sunlight for a short time, the tremendous increase in the colorimetric value caused by steric changes will predominate over any other effect. Thus, it was observed in collaboration with LeRosen, Schroeder, Polgár, and Pauling (116) that very dilute solutions of prolycopene or pro-γ-carotene, which were almost colorless, turned intensely yellow after exposure to sunshine for a few minutes. This change, which in the visible region

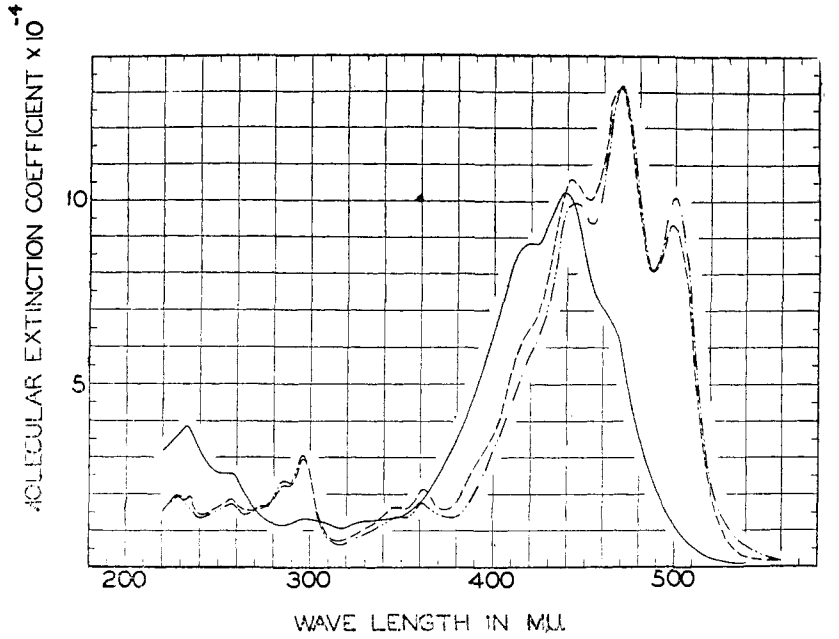


FIG. 4. Photo-isomerization of prolycopene in hexane: ——, fresh solution of prolycopene; — — —, mixture of stereoisomers after insolation for 20 min.; —··—, mixture of stereoisomers after insolation for 60 min. (From J. Am. Chem. Soc. **65**, 1945 (1943))

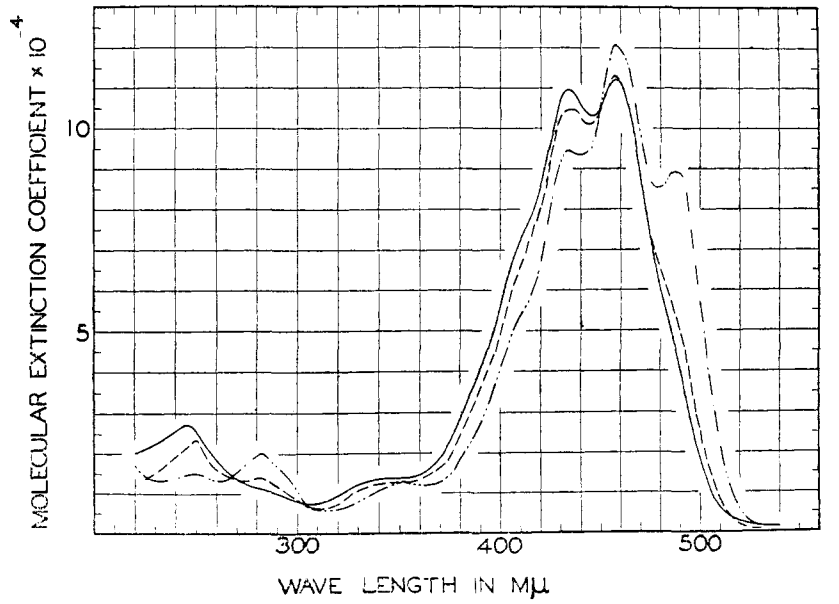


FIG. 5. Photo-isomerization of pro-γ-carotene in hexane: ——, fresh solution of pro-γ-carotene; — — —, mixture of stereoisomers after insolation for 5 min.; —··—, mixture of stereoisomers after insolation for 30 min. (From J. Am. Chem. Soc. **65**, 1944 (1943))

includes both a great increase in the extinction values and a substantial shift toward longer wave lengths, is illustrated in figures 4 and 5 (p. 291).

The photochemical behavior of all-*trans*-α- and -β-carotenes and of their main known isomers is demonstrated in tables 13 and 14 (Polgár and the author (120)). These tables show that the all-*trans* configuration in carotenes is by far the least photosensitive. Some of the neo forms decreased rapidly in quantity upon exposure to sunshine; however, the pigment mixture formed from a comparatively photostable isomer, neo-α-carotene U, still contained 92 per cent of this isomer after irradiation for 45 min. with a 1000-watt Mazda bulb from a distance of 10

TABLE 13

Relative colorimetric values of all-trans-α-carotene and some of its stereoisomers formed by 45 min. insolation (in petroleum ether)

STARTING MATERIAL	RELATIVE COLORIMETRIC VALUES (PER CENT OF THE PIGMENT RECOVERED)								
	Neo U	Neo V	Neo W	Neo X	Neo Y	All-*trans*	Neo A	Neo B	Neo C + D etc.
Neo U	64.5	1.5	3.5	2	3	24		1.5	
Neo V	33	43	4	2		16		2	
Neo W	7.5	5	32.5	1		41	4	7.5	1.5
All-*trans*	1.5		2			93.5		2.5	0.5
Neo B	1.5		34			56.5		8	

TABLE 14

Relative colorimetric values of all-trans-β-carotene and of some of its stereoisomers formed by 45 min. insolation (in petroleum ether)

STARTING MATERIAL	RELATIVE COLORIMETRIC VALUES (PER CENT OF PIGMENT RECOVERED)				
	Neo U	Neo V	All-*trans*	Neo B	Neo C + D etc.
Neo U	36.5		55	6	2.5
All-*trans*	1		98	1	
Neo B	27	2.5	60	5	5.5

cm. After exposure to an ultraviolet lamp (Hanovia, scientific type) for 30 min. the corresponding value was 98 per cent.

That it is not yet possible to correlate structural features with the degree of photosensitivity is demonstrated by the behavior of cryptoxanthin and zeaxanthin. As shown below in table 15, all-*trans*-zeaxanthin, $HOC_{40}H_{54}OH$, in spite of its symmetrical structure, is much more easily bleached by sunshine than is all-*trans*-cryptoxanthin, $C_{40}H_{55}OH$ (Lemmon and the author (113)).

It is advisable to determine by such preliminary experiments the optimum duration of the exposure for the purpose of a study of the photo-isomerization. For cryptoxanthin insolation for 45 min. was found to be adequate (unchanged all-*trans:* neo A = 86:14), and for zeaxanthin insolation for 15 min. Insolation of the latter pigment for 5 min. produced only a slight stereoisomerization; on

the other hand, after exposure for 30 min. and subsequent adsorption analysis, nearly the whole recovered pigment passed into the chromatographic filtrate, under conditions under which any member of the zeaxanthin set would have remained adsorbed in the column. After 15 min. insolation of zeaxanthin (in benzene) the recovered pigment was composed of 48 per cent unchanged all-*trans* form, 11 per cent neozeaxanthins, 11 per cent minor pigments, and 30 per cent of an intensely colored, crystallizable polyene which in the visual spectroscope was similar to zeaxanthin, but on the basis of its partly epiphasic behavior did not belong to that set.

D. SOME PROPERTIES OF CAROTENOIDS CONTAINING *cis* DOUBLE BONDS

The physical character of carotenoids with *cis* double bonds is essentially analogous to that of simpler ethylenic structures.

If an all-*trans* pigment undergoes a steric rearrangement, the color intensity of the solution is decreased and the solubility of the isomers formed is increased. The melting points of crystallizable isomers are lower than that of the all-*trans*

TABLE 15

Loss of color intensity of benzene solutions of cryptoxanthin and zeaxanthin on exposure to sunshine

DURATION OF INSOLATION	LOSS OF COLOR INTENSITY (IN PER CENT OF THE INITIAL VALUE)	
	Cryptoxanthin	Zeaxanthin
minutes		
15	6	21
30	17	39
45	26	63
60	36	88

form. The poly-*cis* compounds, prolycopene and pro-γ-carotene, and some other isomers which contain fewer *cis* double bonds, crystallize readily, but this tendency is markedly diminished, for example, in some members of hydrocarbon sets. Those stereoisomers of α-carotene and β-carotene which are adsorbed below the all-*trans* compound are chromatographically heterogeneous in the sense that fresh solutions of the crystals formed several zones on the column. Such is the situation also with "pseudo-α-carotene" (10), "neocarotene" (11), and "neo-α-carotene" (11). Neolycopene A gave all-*trans* crystals under the conditions employed (129).

Noteworthy is the considerable thermal stability of some stereoisomers when refluxed in solution at 60–80°C. This is evident from various data given below, especially those which are concerned with prolycopene and pro-γ-carotene.

When asymmetric carbon atoms are present, the rotatory power is obviously dependent on *cis–trans* isomerism. The values may undergo alterations in both directions if the all-*trans* molecule is bent. For example, the initial specific levorotation of all-*trans*-zeaxanthin, −42.5°, changed into a dextrorotation upon

either refluxing or iodine catalysis. This inversion proved the presence of a strongly dextrorotatory stereoisomer in the mixture (106). The specific rotation of all-*trans*-gazaniaxanthin, ±0°, increased to + 150° when isomerization of the compound was catalyzed with iodine (125). Some data were also reported by Strain (82).

In several of the sets listed in table 16 one stereoisomer surpasses all the other members of the set tested so far in rotatory power: for example, neozeaxanthin A,

TABLE 16

Dependence of the specific rotatory power on the configuration of some carotenoids

STEREOISOMERIC SET	SOLVENT	MEMBER OF THE SET	$[\alpha]_{Cd}^{25}$ or $[\alpha]_{C}^{20}$	REFERENCES
α-Carotene	Chloroform	All-*trans*	+359°	(120)
		Neo U	+224°	
Gazaniaxanthin....	Petroleum ether	All-*trans*	±0°	(125)
		Neo-Group I	+220°	
Zeaxanthin	Chloroform	All-*trans*	−42.5°	(106)
		Neo A	+120°	
		Neo B	±0°	
Capsanthin.....	Benzene	All-*trans*	±0°	(105)
		Neo A	+89°	
		Neo B	+21°	
		Neo C	+27°	
Capsanthin dipalmitate.....	Petroleum ether	All-*trans*	−30°	(105)
		Neo A	−22°	
		Neo B	−20°	
Capsorubin.....	Benzene	All-*trans*	±0°	(105)
		Neo A	−134°	
		Neo B	−69°	
Capsorubin dipalmitate.....	Petroleum ether	All-*trans*	±0°	(105)
		Neo A	−75°	
		Neo B	−15°	

neocapsanthin A, neocapsorubin A, neocapsorubin dipalmitate A, and the neogazaniaxanthins belonging to Group I. Each of these pigments differs from the all-*trans* form by only a few millimicrons in its visually determined spectral maxima; and furthermore, they adsorb immediately below the all-*trans* compound in the Tswett column.

It remains to be seen whether or not a bending of an all-*trans* carotenoid molecule at or near the center of the chromophore necessarily involves a substantial increase of molecular asymmetry as expressed by increased rotations.

E. *Cis–trans* ISOMERIZATION AND SPECTRAL CHARACTERISTICS

1. Alterations in the visible region by bending of the all-trans molecule

The most spectacular changes caused by *trans* → *cis* rotations of an all-*trans* carotenoid are the alterations of adsorption affinity and light extinction. While, however, the adsorption forces may either increase or decrease as a consequence of *trans* → *cis* rotations, the shift of the spectral maxima in the visible region

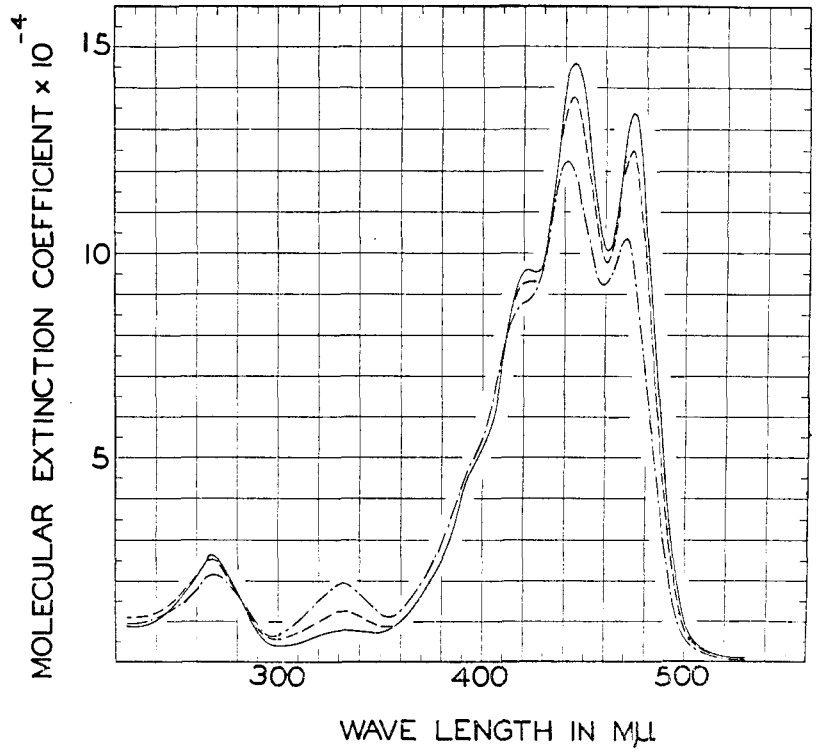


FIG. 6. Molecular extinction curves of α-carotene in hexane: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1522 (1943))

follows a uniform direction in every case. If the solution is concentrated enough, this becomes manifest even to the naked eye by a spectacular decrease in color intensity. The bands observed in the visual spectroscope are then shifted toward shorter wave lengths relative to the corresponding bands of the all-*trans* form. Such a change can be demonstrated by adding a drop of dilute iodine solution to the pigment in a spectroscopic cell while an observer is watching.

Spectroscopic changes in the opposite direction occur if the isomerization of a pigment which has *cis* bonds is catalyzed by iodine. Depending on the con-

figuration, this shift may be larger or smaller than that observed with the all-*trans* form, but in no case will the spectrum of the all-*trans* isomer be reached, since an equilibrium mixture is formed.

Stereoisomerization also causes the molecular extinction curves in the visible region to undergo a characteristic change. If an all-*trans* carotenoid is refluxed or catalyzed, the extinctions decrease. Furthermore, a shift toward shorter wave lengths is observed. The extinction curve of a fresh solution of a carotenoid which has *cis* double bonds, however, is shifted both in position and in intensities in the opposite direction when treated with iodine.

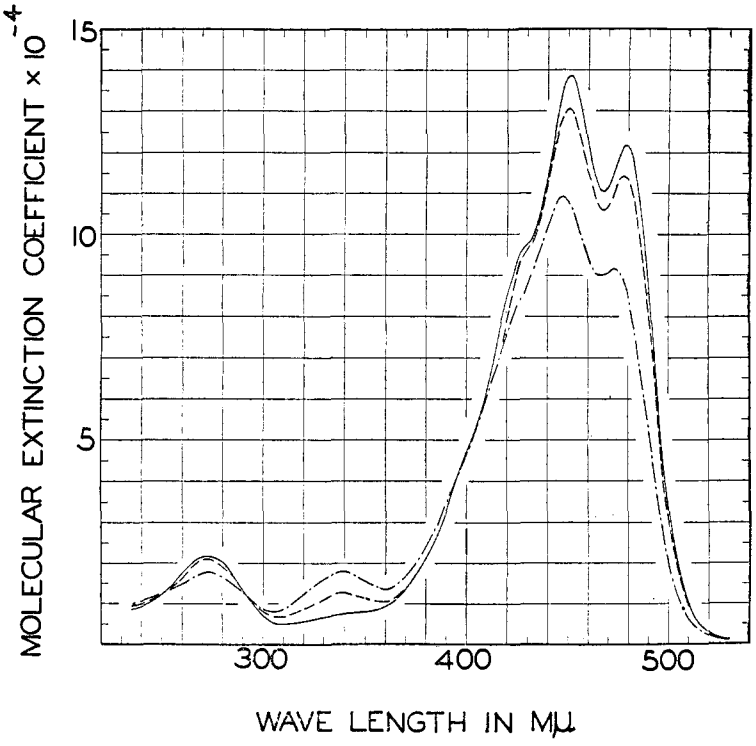


FIG. 7. Molecular extinction curves of β-carotene in hexane: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1523 (1943))

All these observations are in accordance with theoretical treatments presented by Pauling (62, 63), Mulliken (56, 57, 58), and Lewis and Calvin (46).

In a conjugated double-bond system, the single bonds possess sufficient double. bond character to maintain a coplanar system, but these tendencies may be overcome by steric changes. The hydrogen atoms of CH groups adjacent to a double bond overlap somewhat in the *cis* configuration (figure 1) and the consequent interatomic repulsion tends to push the *cis*-isomer out of coplanarity.

This interference with the conjugation shifts the spectral bands to shorter wave lengths (Pauling (62)).

We see that the postulate is well founded both from the experimental and from the theoretical side and that among all members of a stereoisomeric set the all-*trans* compound must be expected to show the greatest light extinction in the visible region.

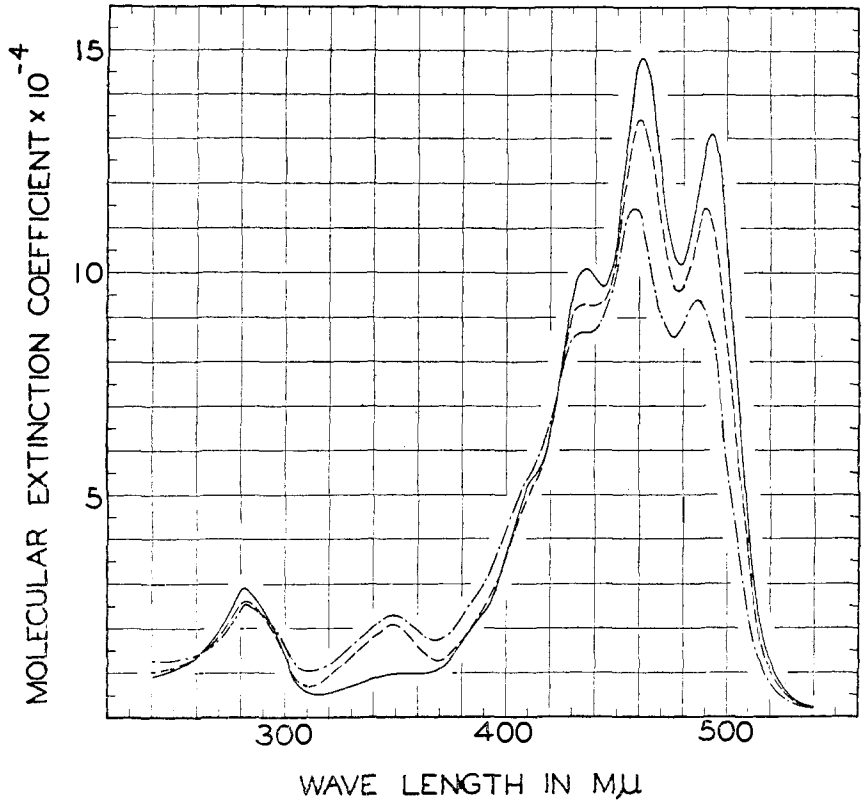


FIG. 8. Molecular extinction curves of γ-carotene in hexane; ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1941 (1943))

Visual spectroscopic data which illustrate the dependence of the position of maxima on the configuration can be found in numerous papers, the first of which was published by Gillam and El Ridi (10). More detailed information for the spectral region down to 380 mμ is provided by the extinction curves of natural and isomerized carotenoids which have been published by Strain (81, 82, 83), Beadle and Zscheile (1), White, Zscheile, and Brunson (97), and by White, Brunson, and Zscheile (96); *cf.* (5, 8, 21a, 21b, 23a, 36, 52a, 54, 55, 73, 76, 130, 131, 132).

2. Alterations in the ultraviolet region by bending of the all-trans molecule: the cis-peak effect

The study of the alterations in spectral curves of carotenoids which attend *trans* → *cis* rotations have been extended farther into the ultraviolet region only recently in collaboration with Polgár (119).

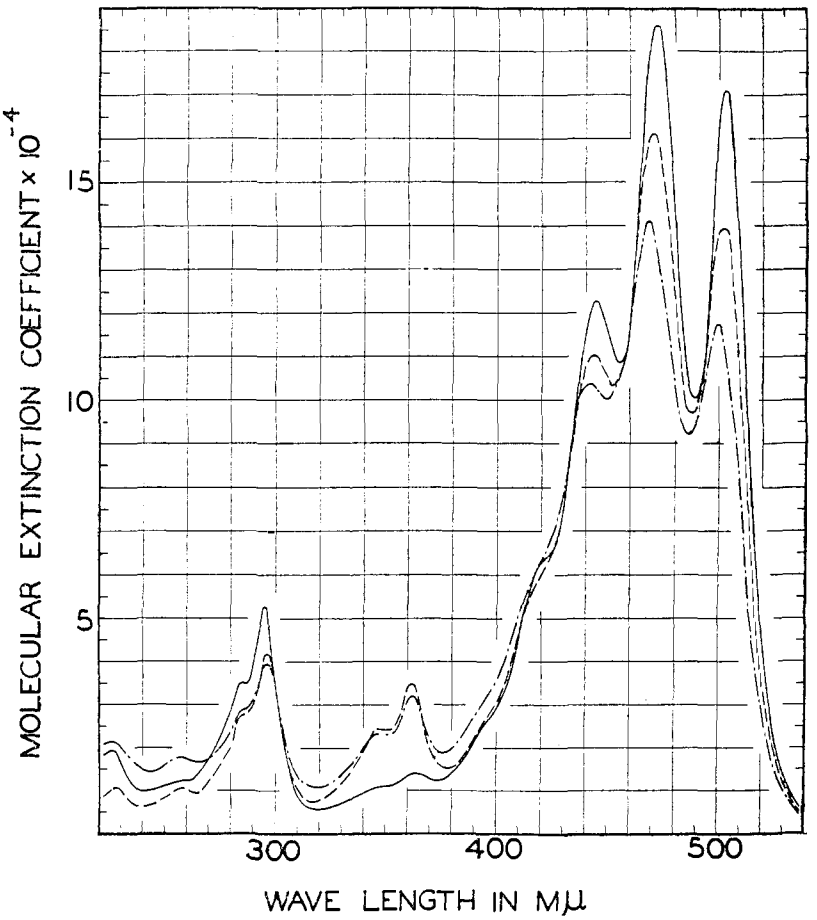


FIG. 9. Molecular extinction curves of lycopene in hexane: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1942 (1943))

It has been customary in our laboratory to characterize each carotenoid studied from the stereochemical viewpoint by three molecular extinction curves which extend if possible to 220 mμ; first, a fresh solution is examined, then the same solution after refluxing for 45–60 min., and finally another portion after standing ¼ to 1 hr. in scattered light (fluorescent lamp) in the presence of iodine (1 to 2 per cent of the pigment). Although the thermal or catalytic treatment

decreases the extinction of an all-*trans* carotenoid in the visual region as well as in the far ultraviolet (260 to 320 mμ in hexane), a new marked maximum develops at a somewhat longer wave-length range in the ultraviolet, that is, somewhere between 320 and 380 mμ where all-*trans* carotenoids show a very slight elevation at most. The new maximum has been termed the "*cis*-peak" and the range of wave lengths in which it appears, the "*cis*-peak region". The differ-

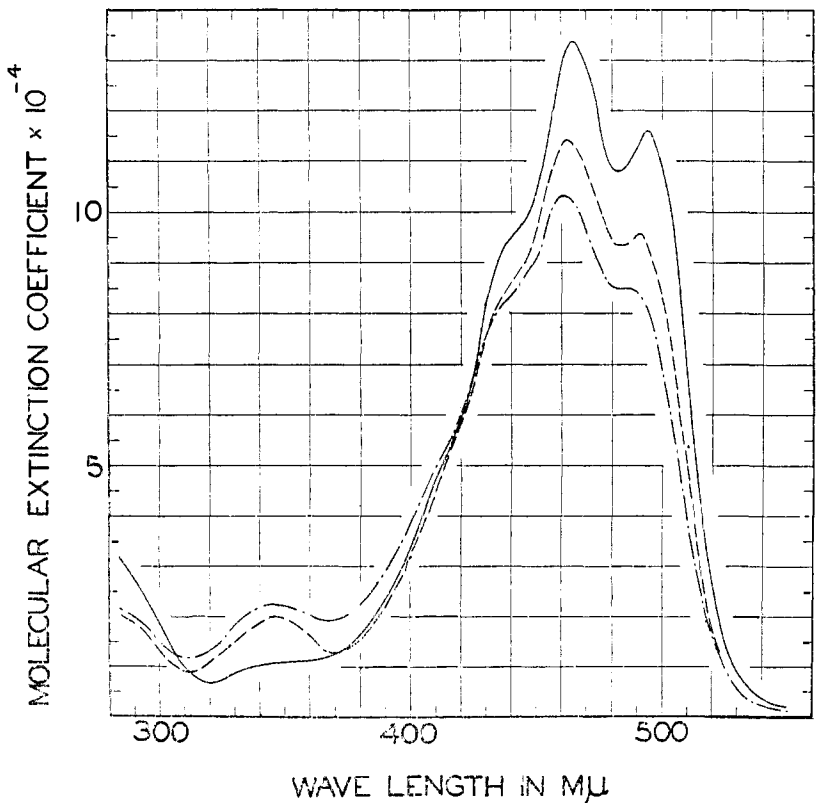


FIG. 10. Molecular extinction curves of cryptoxanthin in benzene: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 318 (1944))

ence between the total extinction of an isomerized pigment and that of its all-*trans* form at the wave length of the peak is the "*cis*-peak effect" (figures 6 to 13).

The *cis*-peak has a definite position in the extinction curve (table 17). The difference between its wave length and that of the longest wave-length maximum of the all-*trans* form is practically a constant, that is, 142 mμ (± 2 mμ) in hexane. It is understandable that the relative position of these two maxima is not markedly altered by the presence of functional groups which are located on the periphery of the molecule.

Maxima observed earlier by McNicholas (50) and by Miller (52, 52a) at wave lengths which correspond to the *cis*-peak were ascribed to oxidation. This was the only reasonable explanation at a time when the stereochemistry of carotenoids was unexplored.

In other cases *cis*-peaks have been represented as a definite feature of the all-*trans* curve, but it is evident that a partial stereoisomerization in the solu-

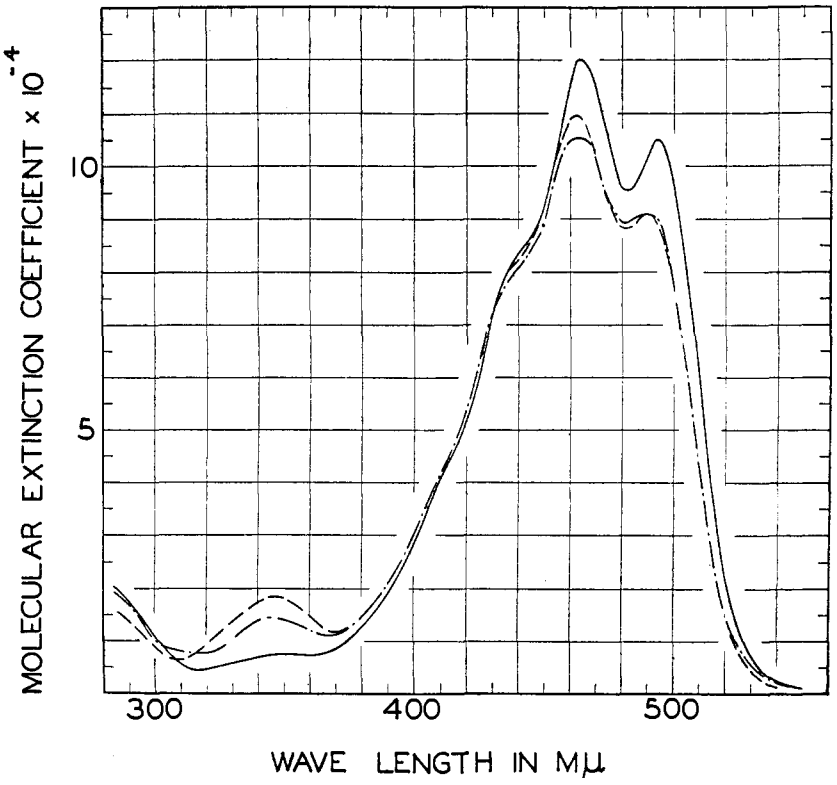


FIG. 11. Molecular extinction curves of zeaxanthin in benzene: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 319 (1944))

tions used was responsible for the effect (*cf.*, for example, some of the figures in reference 23a).

In complete darkness most carotenoids do not develop *cis*-peaks in the presence of iodine within an hour or so. However, a light impulse as short as 5 sec. may be sufficient to produce an effect which is a third or a half of the total peak. Examples are given in figures 14–15 and in table 18 (p. 305). Long illumination in intense light may lead eventually to destructive processes.

The extinction values discussed in the present review refer mainly to benzene,

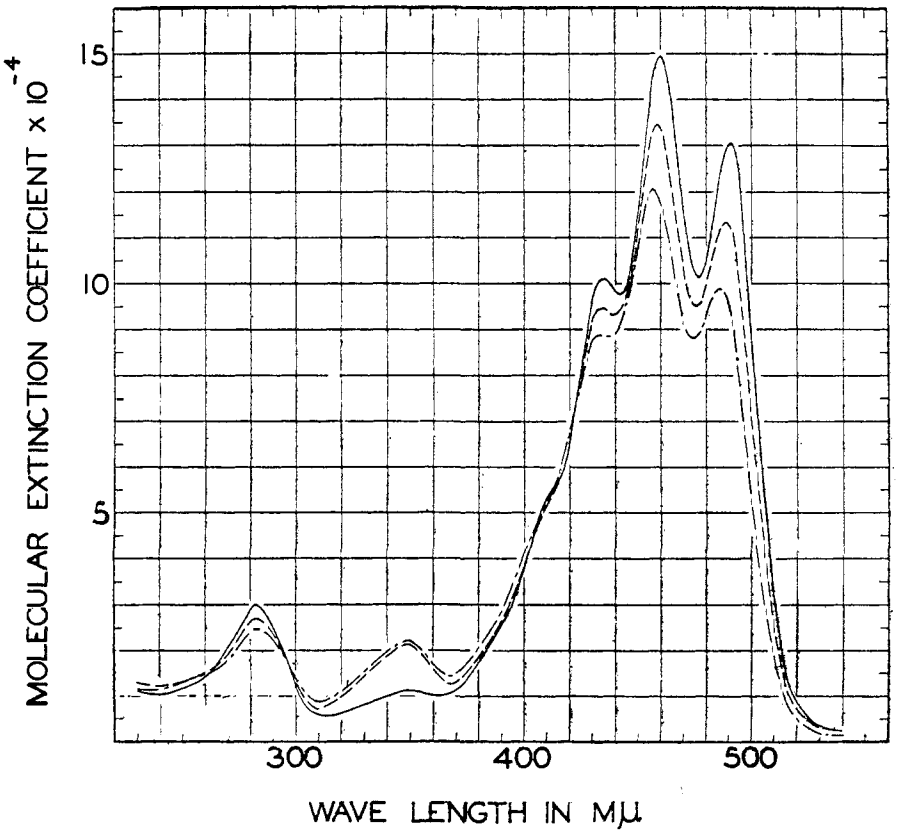


FIG. 12. Molecular extinction curves of gazaniaxanthin in hexane: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65,** 1536 (1943))

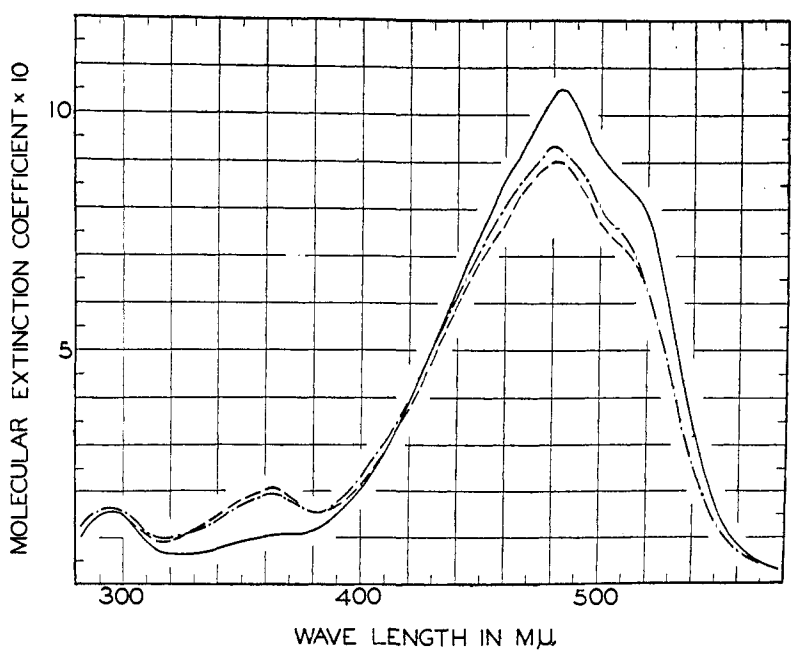


FIG. 13. Molecular extinction curves of capsanthin in benzene: ——, fresh solution of the all-*trans* compound; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66,** 187 (1944))

TABLE 17

Position of the cis-peak in the spectral curve of stereoisomeric equilibrium mixtures of some C_{40}-carotenoids obtained after iodine catalysis (119)

PIGMENT	POSITION OF THE *cis*-PEAK	DISTANCE BETWEEN *cis*-PEAK AND LONGEST WAVE-LENGTH MAXIMUM OF THE ALL-*trans* FORM
	In hexane solution	
	mμ	mμ
5,6-Dihydro-α-carotene (66)	329	140
5,6-Dihydro-β-carotene (66)	331	143.5
α-Carotene	331	143
Lutein	331	143
β-Carotene	339.5	139.5
Cryptoxanthin	339	141
Physalien	338	141
γ-Carotene	349.5	143.5
Gazaniaxanthin (71, 125)	349	142
Lycopene	362	141.5
Capsanthin	355	143
Celaxanthin (44)	380	144
	In benzene solution	
Cryptoxanthin	348	146
Zeaxanthin	348	145
Capsanthin	363	145

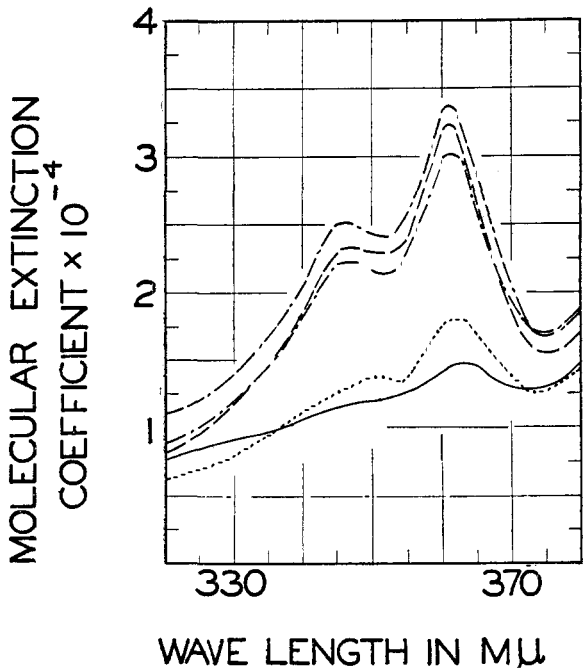


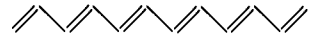
FIG. 14. Influence of illumination on the development of the *cis*-peak in an iodine-catalyzed solution of all-*trans*-lycopene in hexane: · · · · , molecular extinction curve after zero seconds; — —, after 5 sec.; — — · — —, after 30 sec.; — · —, after 15 min. illumination. The full line denotes the curve taken before the addition of iodine, without illumination. (From J. Am. Chem. Soc. **66**, 189 (1944))

petroleum ether, or hexane solutions. Lycopene (and some other pigments) when refluxed or catalyzed with iodine in carbon disulfide show elevated but remarkably flat extinction curves in the *cis*-peak region (unpublished work by Polgár and the author).

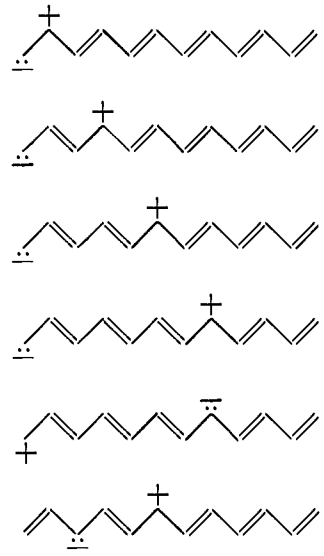
3. Interpretation of the cis-peak effect

The following theoretical considerations have recently been given by Pauling *et al.* (116) and are partly based on earlier treatments (46, 47, 56, 57, 58, 62, 63, 64).

The highly unsaturated chromophore in carotenoids may be discussed in terms of a system resonating among the conventional structure



and a great number of ionic structures, such as:



The conventional structure makes the most important contribution to the normal state, whereas the ionic structures contribute in the main to excited states.

As we have seen, in the extinction curves of carotenoids three main regions must be differentiated, also from the practical viewpoint of the spectroscopist, *viz.*: (*1*) the region of the extraordinarily strong extinction in the visible region, which constitutes the fundamental absorption band, having a more or less pronounced vibrational fine structure; (*2*) the "*cis*-peak region" in the near ultraviolet (first overtone); and (*3*) a region in the far ultraviolet (second overtone). It is assumed that these absorption bands result from transitions from the normal electronic state to the three excited states, to wit, $0 \rightarrow 1$, $0 \rightarrow 2$, and $0 \rightarrow 3$, respectively. Following Lewis and Calvin (46) we may compare these with the classical modes of vibration of mobile "unsaturation" electrons along the chain.

The observed spectral bands can be correlated with the following manners of oscillation:

Fundamental band: The electrons tend to concentrate first near one end and then near the other of the conjugated system. 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 dipole moment.

Band in the "cis-peak region": This results from the oscillation of the electrons from the two ends of the conjugated system toward the middle and from the middle toward the two ends.

Band in the far ultraviolet: This involves concentration of the electrons alternately in the first and third and the second and fourth quarters of the system.

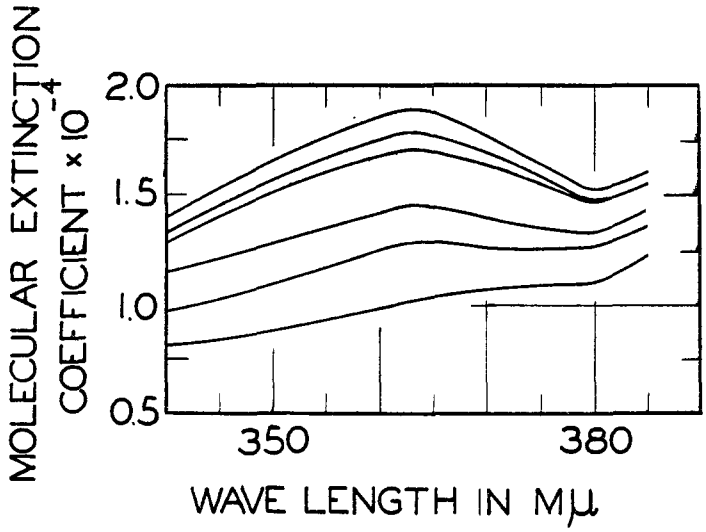


FIG. 15. Influence of illumination on the development of the *cis*-peak in an iodine-catalyzed solution of all-*trans*-capsanthin in benzene. Molecular extinction curves from bottom to top: after 0 sec., 5 sec., 30 sec., 2.5 min., 15 min., and 30 min. illumination. (From J. Am. Chem. Soc. **66**, 188 (1944))

The intensity of this maximum corresponding to the transition $0 \rightarrow 3$ may be expected to vary in rough proportionality to that of the fundamental band, and this is confirmed by experiments.

The intensity of a spectral band is proportional to the square of the corresponding dipole moment, and hence essentially to the square of the length of the conjugated system (57, 58, 62). Consequently, the maximum intensity of the fundamental band would be shown by the all-*trans* member of a set. All stereoisomers which have a vertical plane of symmetry have a distance between the two ends of the conjugated system smaller than the all-*trans* isomer by the factor $\cos \alpha$, with $\alpha = 27°22'$ (if the carbon bond angle along the chain is 125°16'). The intensities for all stereoisomers permitted by the given postulates should lie between these extremes.

TABLE 18

Influence of light on the development of the cis-peak in iodine-catalyzed solutions of all-trans carotenoids (67, 113, 120)

(Mazda fluorescent lamp at 60 cm. distance)

PIGMENT	DURATION OF THE ILLUMINATION	MOLECULAR EXTINCTION COEFFICIENT AT THE LONGEST WAVE-LENGTH MAXIMUM $E^{mol.}_{1cm.} \times 10^{-4}$	MOLECULAR EXTINCTION COEFFICIENT AT THE *cis*-PEAK WAVE LENGTH $E^{mol.}_{1cm.} \times 10^{-4}$	DIFFERENCE OF THE MOLECULAR EXTINCTION COEFFICIENT FROM THE 0 SEC. VALUE, EXPRESSED IN PER CENT OF THE GREATEST CHANGE OBSERVED IN EACH SET
α-Carotene (in hexane)...	0 sec.	14.6	0.8(6)	0
	30 sec.	13.7	1.94	75
	2½ min.	13.4	2.11	87
	15 min.	13.2	2.16	90
	30 min.	12.7	2.30	100
	60 min.	11.3	2.26	97
β-Carotene (in hexane) ...	0 sec.	14.2	0.8(7)	0
	30 sec.	13.2	1.95	80
	2½ min.	13.0	2.04	87
	15 min.	12.3	2.22	100
	30 min.	10.9	2.22	100
Lycopene (in hexane)....	0 sec.	18.6	1.47	0
	5 sec.	15.5	3.2	92
	30 sec.	15.6	3.4	100
	2½ min.	15.4	3.2	92
	15 min.	14.0	3.0	81
Cryptoxanthin (in benzene).....	0 sec.	13.4	0.70	0
	5 sec.	13.1	0.81	10
	30 sec.	12.7	1.16	42
	2½ min.	12.0	1.62	84
	15 min.	11.0	1.77	98
	30 min.	10.0	1.79	100
Zeaxanthin (in benzene)..	0 sec.	12.0	0.89	0
	5 sec.	11.8	1.25	42
	30 sec.	11.4	1.47	68
	2½ min.	11.1	1.64	88
	15 min.	10.8	1.74	100
	30 min.	10.5	1.71	97
	60 min.	10.1	1.74	100
Capsanthin (in benzene)..	0 sec.	9.9	1.02	0
	5 sec.	9.8	1.29	31
	30 sec.	9.5	1.47	51
	2½ min.	9.3	1.72	80
	15 min.	9.0	1.80	89
	30 min.	9.0	1.89	99
	60 min.	8.9	1.90	100

The nature of the $0 \rightarrow 2$ oscillation, which was discussed by Mulliken (57, 58), 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 with a center of symmetry. However, that isomer which has one centrally located *cis* double bond possesses a dipole moment for the transition $0 \rightarrow 2$ which is perpendicular to the straight line between the ends of the conjugated system instead of parallel to it. This isomer would show the highest *cis*-peak. As a rough approximation the intensity of the *cis*-peak of any member of a stereoisomeric set 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 ends. Accordingly, only a few of the stereoisomers possible can have *cis*-peaks approaching in height that of the isomer with one *cis* bond in the center.

If these theoretical viewpoints are correct and if we start at the center of an all-*trans* chromophore and perform successive *trans* $\rightarrow$ *cis* rotations, then the *cis*-peak must first rise and then fall again as the molecule bends and straightens out. A continuous rise of the peak during these rearrangements is not to be expected.

Starting materials for an experimental test of these two alternatives cannot yet be prepared artificially, since poly-*cis* compounds have not been observed when a pigment is isomerized by current methods. However, nature has provided two such compounds, prolycopene (45) and pro-γ-carotene (122), which have already been discussed. In the visible region, petroleum ether solutions of these pigments show maxima at 35 mμ and 31 mμ shorter wave lengths than those of the maxima of the corresponding all-*trans* pigments, lycopene and γ-carotene.

It was first assumed (117) that prolycopene is an all-*cis* isomer, but melting of its crystals produced a new pigment with still shorter wave-length maxima in the visual region; therefore, the all-*cis* configuration is claimed for the latter pigment. It is very probable that six of the seven stereochemically available double bonds are *cis* in prolycopene and five of the six such bonds in pro-γ-carotene. The extent of the visually observed spectral shift in the last possible *trans* $\rightarrow$ *cis* rotation is approximately the same as observed in the first step. In petroleum ether solutions the following longest wave-length maxima were measured ($\pm$ 1 mμ):

All-*trans*-lycopene	504.5 mμ	Δ = 4.5 mμ
Neolycopene A	500	
Prolycopene	470.5	Δ = 4.5 mμ
All-*cis*-lycopene	466	

Experiments carried out in collaboration with LeRosen, Schroeder, Polgár, and Pauling (116) showed that the extinction curves of the pro-carotenoids and of all-*cis*-lycopene are nearly as flat in the *cis*-peak region as the curve of the all-*trans* form (figures 16 to 18). These curves confirm that the theoretical viewpoints suggested are essentially correct and that a bending of the all-*trans* molecule, especially in the middle of the conjugated system, is mainly responsible for the appearance of a high *cis*-peak.

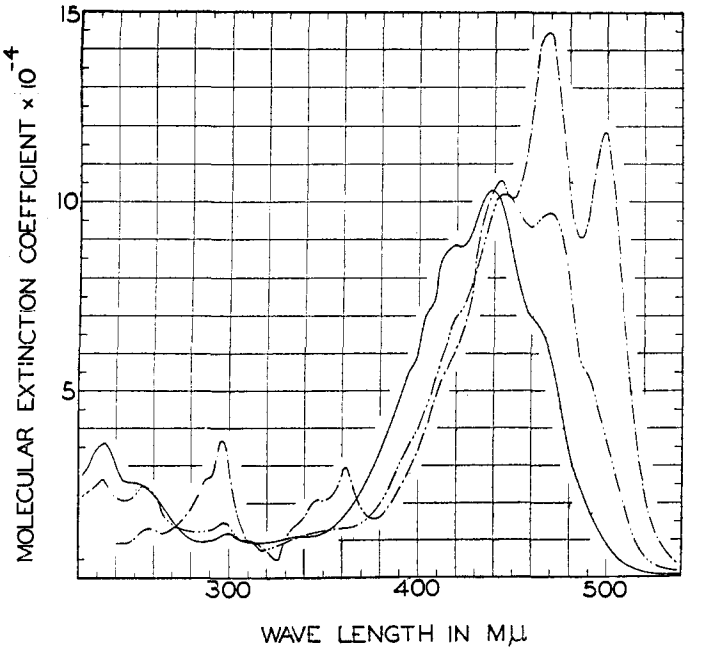


FIG. 16. Molecular extinction curves of prolycopene and an unnamed crystalline stereoisomer in hexane: ——, fresh solution of prolycopene; —··—, fresh solution of the isomer; —·—, mixture of stereoisomers in the prolycopene solution after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1943 (1943))

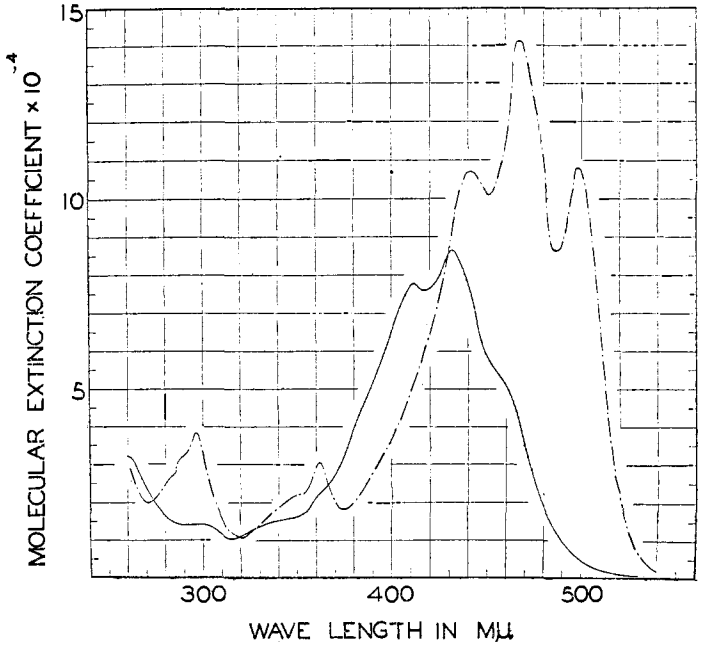


FIG. 17. Molecular extinction curves of all-*cis*-lycopene in hexane: ——, fresh solution of all-*cis*-lycopene; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1943 (1943))

The spectral phenomena which can be used for a rapid diagnosis of a given stereochemical situation are summarized below in table 19.

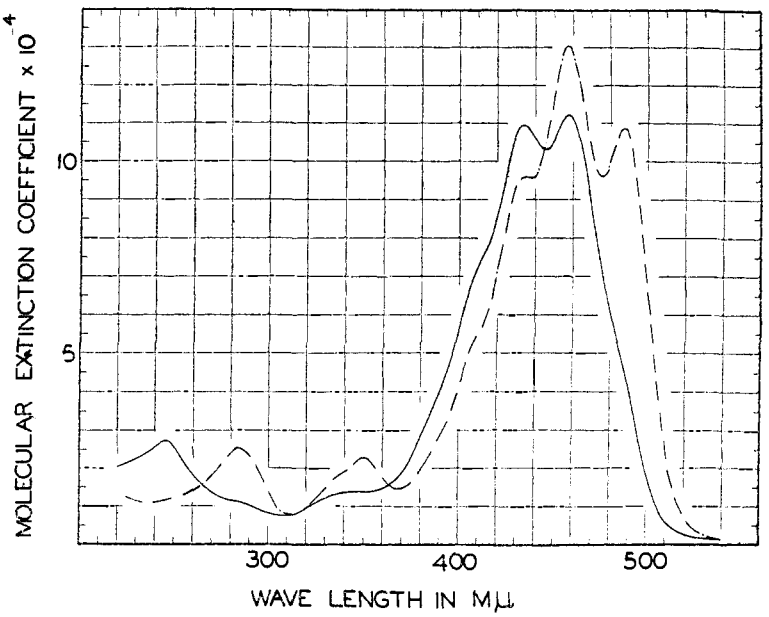


FIG. 18. Molecular extinction curves of pro-γ-carotene in hexane: ——, fresh solution of pro-γ-carotene; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1941 (1943))

TABLE 19

Types of spectroscopic phenomena in the cis-peak and visible regions observed upon iodine catalysis

CONFIGURATION	SHAPE OF THE EXTINCTION CURVE IN THE *cis*-PEAK REGION	CHANGE OF EXTINCTION UPON IODINE CATALYSIS	
		In the visible region	In the *cis*-peak region
All-*trans*	Flat	Decrease	Increase
Central mono-*cis*	High peak	Increase	Decrease
All-*cis* or all-but-one-*cis*	Flat	Great increase	Increase

F. ASSIGNMENT OF CONFIGURATIONS

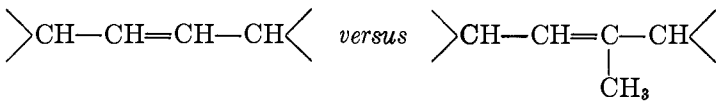
We shall now investigate to what extent it is possible at present to assign definite spatial structures to stereoisomers of carotenoids. This task is two-fold: in each case the number of the *cis* double bonds must be found and their position in the chromophore must be determined. Two different approaches to such work will be now discussed: first, considerations based on thermal (or photochemical) stereoisomerization which have so far been employed to only a

small extent, and second, considerations based on spectral phenomena, which constitute the main basis for the assignment of configurations.

1. Thermal and photochemical isomerization methods

Some postulates concerning these methods have recently been discussed (116, 120).

According to the theory of conjugated systems, each of the double bonds of the system loses some of its double-bond character to the adjoining single bonds, the amount lost increasing from the ends toward the center. Hence, in a system containing an odd number of conjugated double bonds the central double bond has the smallest amount of double-bond character. In carotenoids this effect may possibly be enhanced by the structural difference between the central double bond and the other double bonds:



Because the activation energy for *trans* → *cis* isomerization about the central double bond is less than about the other double bonds, all members of a given set may be classified into "epimeric" pairs, the members of which are *cis* and *trans*, respectively, about the central double bond but otherwise possess the same configuration. The members of each pair are especially easily interconvertible, and the central-*trans*-isomer of each pair is more stable thermodynamically than its central-*cis*-isomer. For each pair the thermal isomerization

central-*trans* ⇄ central-*cis*

is expected to occur readily at lower temperatures than isomerizations involving other double bonds. Since the activation energies for forward and reverse reactions differ by the heat of the reaction, the less stable isomer of each pair (the central-*cis* compound) should isomerize at lower temperatures than the other member of the pair (116).

It remains to be seen how extensively such predictions can be used in practical experimentation. In any case, the above conclusions are in agreement with the observed appearance of a main isomer which is produced upon isomerization of an all-*trans* carotenoid and which possesses the highest *cis*-peak in the set.

Another use of thermal or photochemical methods is possible when a moderate heating or illumination yields mainly another member of the set. For example, if a mono-*cis* compound forms mainly a certain di-*cis* compound, it is reasonable to assume that the two pigments have one *cis*-position in common. An example to illustrate this method will be found in Part III under bixin (page 329).

2. Methods based on spectra

The spectroscopic phenomena which form the basis for the assignment of configurations take place partly in the visible and partly in the ultraviolet region.

(*a*) *Investigations in the visible region:* Since the visual maxima of all members of a stereoisomeric set are located at shorter wave lengths than the corresponding maxima of the all-*trans* compound, this difference, if correctly interpreted, should give us the number of the *cis* bonds in each case. The main difficulty lies in the lack of a reliable theoretical basis which would show the precise relation between this difference and the position of a *cis* double bond in the long unsaturated system. It may well be that the decrease in wave length caused by one *trans* → *cis* rotation is smaller for a peripheral double bond than for one situated at or near the center of the system. In practice, however, no essential differences of this character have been noticed up to the present time.

According to probability, when an all-*trans* compound undergoes isomerization the formation of mono-*cis* pigments will be favored. The main isomer (or one of the two main isomers) formed possesses a visually observed spectrum in petroleum ether or benzene in which the position of the longest wave-length maximum has been shifted 5 mμ ($\pm$ 1 mμ) relative to the maximum of the corresponding all-*trans* compound. It is safe to assume that this regularly observed minimum difference is caused by one *trans* → *cis* rotation. Stereoisomers with about 10 mμ difference should be classified as di-*cis* compounds, etc. On this basis, by using the values listed in table 21 (third column), it is possible to differentiate between all-*trans*, mono-*cis*, and di-*cis* members of a set, even if some doubtful cases may arise. If the spectral difference is considerably greater than 10 mμ, the insecurity increases, because nothing definite is known about the additivity of spectral differences and their dependence on the relative position of the double bonds concerned.

In principle, some configurations may also be derived from the relative intensities of the highest extinctions of each member of a set.

This principle has been used by Pauling (62), for example, in the case of lycopene and neolycopene. Pauling pointed out that the integrated absorption coefficients should be approximately proportional to the squares of the actual distances between the ends of the conjugated system. The all-*trans* member possesses the highest values in each set. An application of this principle will be given in Part III for methylbixin (p. 332).

(*b*) *Investigations in the cis-peak region:* The *cis*-peak effect was first observed in complicated stereoisomeric mixtures in which the unchanged portion of the all-*trans* compound amounted to the half or more of the total pigment. Consequently, the rest of the mixture, composed of a number of *cis* compounds, must show a higher molecular extinction value at the peak wave length than the entire equilibrium mixture.

A resolution of the effects can be conveniently carried out by chromatographic analysis of the mixture and the determination of the molecular extinction of each isomer at the *cis*-peak wave length. The first experiment of this kind, with Polgár (119), showed that those members of the β-carotene set which, on the Tswett column, are adsorbed below the all-*trans* pigment are responsible for the major fraction of the peak observed upon iodine catalysis. Further investigation has proved that generally one or two main isomers are responsible for the *cis*-peak observed in an isomerization mixture (table 20).

A characteristic constant of each stereoisomer is its "*cis*-peak effect," i.e., the difference between its molar extinction and that of the all-*trans* form at the *cis*-peak wave length (table 21, fifth column, p. 312).

In the following we intend to give a stereochemical survey of some carotenoid sets. The author does not claim that the difficult problem of the assignment of configurations has reached a final solution. However, the phenomena observed permit a reasonable stereochemical discussion in several cases for which formerly no experimental data whatsoever were available.

It will be practical to consider separately the carotenoid sets which contain an odd member of conjugated double bonds and those which contain an even number of such bonds.

The stereochemistry of the former subclass is simpler, especially if the molecule is symmetrical (e.g., β-carotene). In this case, we can speak of a truly "central" double bond. For any non-central double bond of the system, there

TABLE 20

Stereoisomers responsible for the major part of the cis-peak effect of the total equilibrium mixture obtained by iodine catalysis

STEREOISOMERIC SET	MEMBER OF THE SET	APPROXIMATE PERCENTAGE OF THE MEMBER IN THE EQUILIBRIUM	APPROXIMATE PERCENTAGE OF THE TOTAL *cis*-PEAK EFFECT CAUSED BY THE MEMBER	REFERENCES
α-Carotene	Neo B	13	55	(120)
β-Carotene	Neo B	25	75	(65)
Lycopene	Neo A	30–40	95	(128)
Lutein	Neo A	17	70	(128)
Cryptoxanthin	Neo A	23	60	(113)
Zeaxanthin	Neo A + Neo B	30	90	(106)
Capsanthin	Neo A	20	80	(105)

is another which is stereochemically equivalent. If n sterically effective double bonds are present, $(n - 1)/2 + 1$ mono-*cis* isomers are expected.

The situation is more complicated if the number of conjugated double bonds is even (e.g., α-carotene). In this case, the center of the chromophore is a single bond and there are two "central" double bonds. Considering the structure of carotenoids, the system is then non-symmetrical, and the expected number of mono-*cis* compounds is equal to that of the sterically effective double bonds.

3. Stereochemical sets with an odd number of conjugated carbon–carbon double bonds

(a) Lycopene set (figures 16, 17, 19–21) (116, 120)

The number of possible stereoisomers is seventy-two. The chromophore is structurally and stereochemically symmetrical and contains seven effective double bonds of which *1* and *11*, *3* and *9*, and *5* and *7* have equivalent positions. This set was used by Pauling and our group to develop a theory of the *cis*-peak effect.

TABLE 21

Typical spectroscopic data and the cis-peak effect in some stereochemical sets of carotenoids (in the sequence of decreasing adsorption affinities within the sets)

STEREOISOMERIC SET	MEMBER OF THE SET	DIFFERENCE BETWEEN THE VISUALLY ESTABLISHED LONGEST WAVE-LENGTH MAXIMUM OF THE MEMBER AND THAT OF THE ALL-*trans* FORM (IN PETROLEUM ETHER)	MOLECULAR EXTINCTION COEFFICIENT AT THE *cis*-PEAK WAVE LENGTH $E^{mol.}_{1\,cm.} \times 10^{-4}$	DIFFERENCE OF $E^{mol.}_{1\,cm.} \times 10^{-4}$ FOR MEMBER OF THE SET AND ALL-*trans* FORM AT THE *cis*-PEAK WAVE LENGTH	REFERENCES
		mμ			
α-Carotene (in hexane)	Neo U	5.5	1.2	0.4	(120)
	Neo V	11.5	1.1	0.3	
	Neo W	6.5	1.6	0.8	
	Neo X	13.5	2.7	1.9	
	All-*trans*	0	0.8	0	
	Neo A	8.5	3.8	3.0	
	Neo B	10.5	3.8	3.0	
	Neo C	4.5	4.5	3.7	
β-Carotene (in hexane)	Neo U	5	1.3	0.5	(120)
	Neo V	13.5	0.8	0	
	All-*trans*	0	0.8	0	
	Neo B	10.5	3.4	2.6	
	Neo E	8.5	3.4	2.6	
γ-Carotene (in hexane)	All-*trans*	0	0.95	0	(116)
	Neo-γ-carotene	5			
	Pro-γ-carotene	31	1.3	0.35	
Lycopene (in hexane)	All-*trans*	0	1.4	0	(116, 120)
	Neo A	5	6.8	5.4	
	Neo B	8	3.7	2.3	
	Unnamed crystaline isomer	28	1.3	−0.1	
	Prolycopene	34	1.6	0.2	
	All-*cis*	38.5	2.2	0.8	
Cryptoxanthin (in hexane)	Neo U	5	1.5	0.3	(113)
	All-*trans*	0	1.2	0	
	Neo A	6.5	4.2	3.0	
	Neo B	4.5	4.5	3.3	
Cryptoxanthin (in benzene)	Neo U	5	1.7	0.7	(113)
	All-*trans*	0	1.0	0.7	
	Neo A	6.5	3.4	2.4	
	Neo B	4.5	4.6	3.6	

TABLE 21—*Continued*

STEREOISOMERIC SET	MEMBER OF THE SET	DIFFERENCE BETWEEN THE VISUALLY ESTABLISHED LONGEST WAVE-LENGTH MAXIMUM OF THE MEMBER AND THAT OF THE ALL-*trans* FORM (IN PETROLEUM ETHER)	MOLECULAR EXTINCTION COEFFICIENT AT THE *cis*-PEAK WAVE LENGTH $E^{mol.}_{1cm.} \times 10^{-4}$	DIFFERENCE OF $E^{mol.}_{1cm.} \times 10^{-4}$ FOR MEMBER OF THE SET AND ALL-*trans* FORM AT THE *cis*-PEAK WAVE LENGTH	REFERENCES
		mμ			
Zeaxanthin (in benzene).....	Neo A	5.5	4.4	3.7	(113)
	Neo B	5.5	2.4	1.7	
	Neo C	8.5	3.9	3.2	
	All-*trans*	0	0.7	0	
Lutein (in benzene) .	Neo A	6	4.9	4.1	(120)
	Neo B	7	2.1	1.3	
	All-*trans*	0	0.8	0	
Capsanthin (in benzene).....	Neo A	6	4.4	3.4	(67)
	Neo B	6	2.7	1.6	
	Neo C	10.5	1.9	0.8	
	All-*trans*	0	1.1	0	

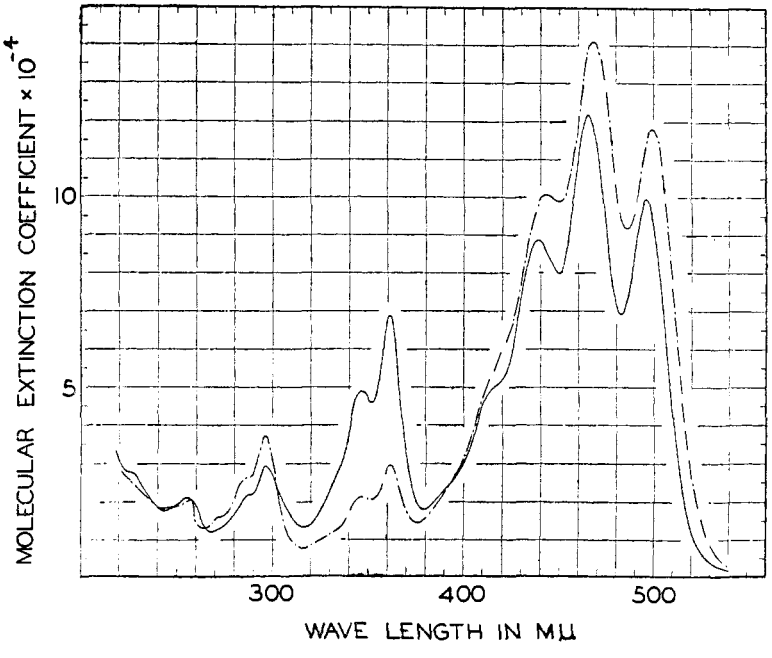


FIG. 19. Molecular extinction curves of neolycopene A in hexane: ——, fresh solution of neolycopene A; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1944 (1943))

The main isomer observed in chromatograms is neolycopene A, which has the highest *cis*-peak so far measured in any set. On the basis of this characteristic and the spectral difference of 4.5 mμ (table 21), this isomer must be *6*-mono-*cis*-lycopene. Neolycopene B is a di-*cis* isomer which may well be *1,6*- or *3,6*-di-*cis*-lycopene. It has been mentioned earlier that prolycopene is very probably an all-but-one-*cis* isomer. We assume that its correct designation is *1,3,5,7,9,11*-hexa-*cis*-lycopene. Its relatively high thermostability is explained by the effect of the many peripheral *cis* double bonds which decrease the conjugation

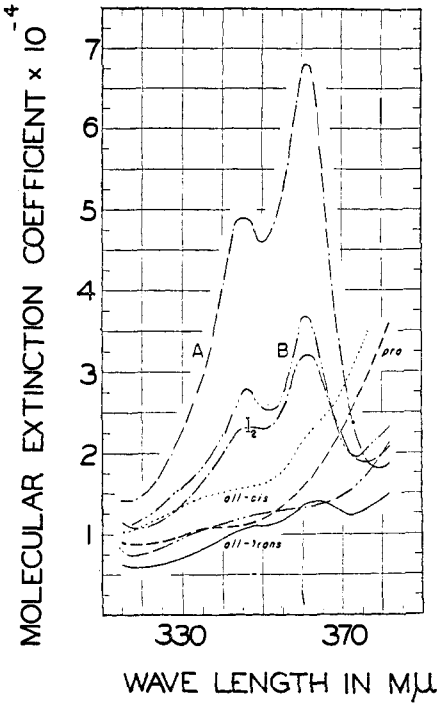


FIG. 20. Molecular extinction curves of some members of the stereoisomeric lycopene set in the *cis*-peak region in hexane: ··—— ·· denotes an unnamed crystalline isomer; I_2 indicates the equilibrium mixture obtained upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 141 (1944))

and stabilize the centrally located *trans* double bond, the only stereochemically effective double bond which remained in *trans* configuration. All-*cis*-lycopene should be termed *1,3,5,6,7,9,11*-hepta-*cis*-lycopene.

(b) β-Carotene set (figures 2, 22, 23) (120)

The number of possible stereoisomers is twenty. The chromophore is structurally and stereochemically symmetrical and contains five effective double bonds, of which *3* and *9* as well as *5* and *7* have equivalent positions. All stereoisomers of this set are represented in figure 2 (on p. 279) by their carbon skeletons.

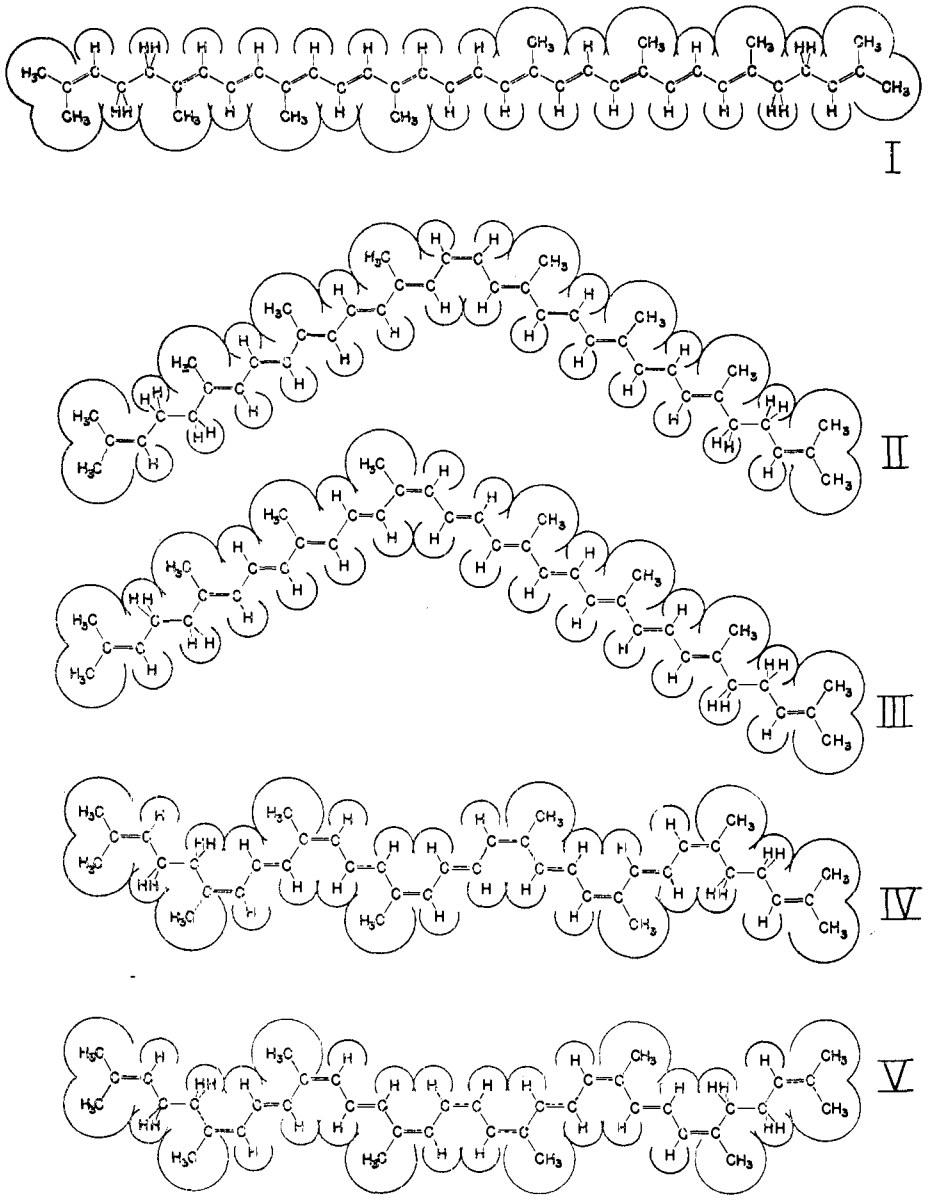


FIG. 21. Suggested stereochemical structures of some members of the lycopene set: I, all-*trans*-lycopene; II, neolycopene A; III, *5-cis*-lycopene; IV, prolycopene; V, all-*cis*-lycopene. (From J. Am. Chem. Soc. **65**, 1946 (1943))

The positions of the visual maxima show that neo-β-carotene U is a mono-*cis*, and neo V a di-*cis* compound. Neither can contain a *6-cis* central double bond because of their low *cis*-peaks and relatively high thermostability when refluxed.

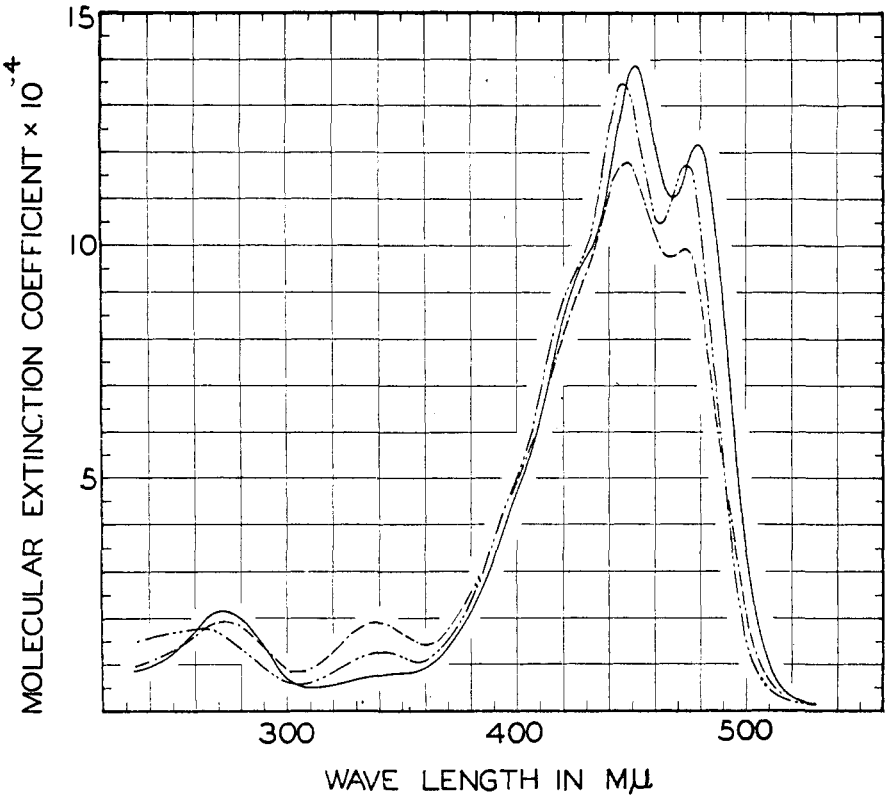


FIG. 22. Molecular extinction curves of neo-β-carotene U, compared with that of all-*trans*-β-carotene, in hexane: ——, β-carotene; —··—, fresh solution of neo-β-carotene U; ——·——, mixture of stereoisomers obtained from neo U upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **65**, 1524 (1943))

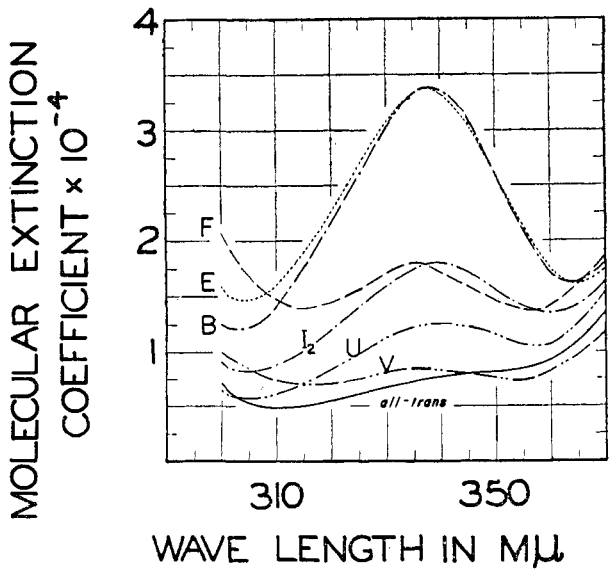


FIG. 23. Molecular extinction curves of some members of the stereoisomeric β-carotene set in the *cis*-peak region in hexane. I_2 indicates the equilibrium mixture obtained upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 139 (1944))

The most probable configurations are: neo-β-carotene U = *3*-mono-*cis*-β-carotene, and neo-β-carotene V = *3,9*-di-*cis*-β-carotene. However, a *5,7*-di-*cis* configuration cannot be excluded in the latter case.

A main isomer observed in chromatograms, neo-β-carotene B, can be interpreted as a di-*cis*-β-carotene which has one of its *cis*-bonds in the central position; such an assignment is substantiated by the high *cis*-peak and the relatively slight thermostability. Since a comparison of the *cis*-peak with the models excludes the *5,6*-di-*cis* configuration, the best assumption is that neo-β-carotene B is

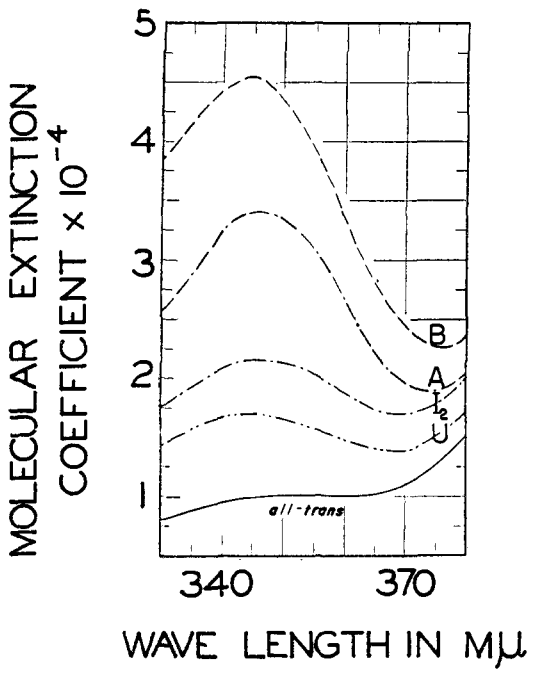


FIG. 24. Molecular extinction curves of some members of the stereoisomeric cryptoxanthin set in the *cis*-peak region in benzene. I_2 indicates the equilibrium mixture obtained upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 320 (1944))

3,6-di-*cis*-β-carotene. The minor isomer, neo E, seems to belong to the same type, but because of its lability no definite spatial structure has been suggested.

(c) Cryptoxanthin set (figure 24) (113)

The number of possible stereoisomers is thirty-two. The chromophore is stereochemically symmetrical but structurally unsymmetrical because of the presence of a hydroxyl group at only one end of the molecule. All five effective double bonds, *viz.*, *3,5,6,7*, and *9*, may give rise to different mono-*cis* isomers.

Evidently, the neo forms U and B are mono-*cis* compounds. The enormous difference in their *cis*-peaks suggests that neocryptoxanthin U is *3*- or *9*-mono-*cis*-cryptoxanthin and neocryptoxanthin B is *6*-mono-*cis*-cryptoxanthin. The

configuration of neo A is somewhat uncertain because its relatively high *cis*-peak and the spectral difference of 6.5 mμ permit several interpretations. This isomer could be a di-*cis* compound with one central and one peripheral double bond in *cis* configuration.

(d) Zeaxanthin set (figure 25) (113)

The general characteristics are the same as given for the β-carotene set.

The best interpretation for the two main isomers, which cannot be differentiated in the visual spectroscope but which have different *cis*-peaks, is: neozeaxanthin A = *6*-mono-*cis*-zeaxanthin and neozeaxanthin B = *5*-mono-*cis*-zeaxanthin. The peak of the minor isomer C is very high, and its spectral

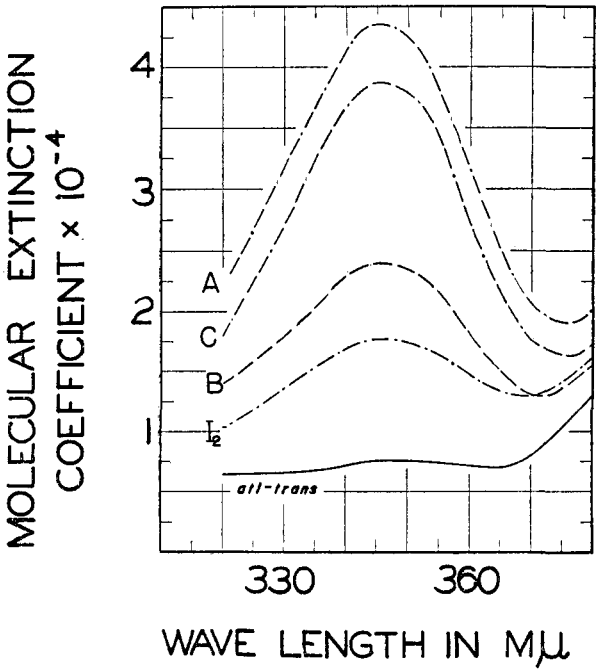


FIG. 25. Molecular extinction curves of some members of the stereoisomeric zeaxanthin set in the *cis*-peak region in benzene. I_2 indicates the equilibrium mixture obtained upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 320 (1944))

difference in the visible region is 8.5 mμ. This isomer could be a di-*cis* compound with one *cis* double bond in the center, *viz.*, *3,6*-di-*cis*-zeaxanthin.

(e) γ-Carotene set (figures 5, 8, 18) (116)

The number of possible stereoisomers is sixty-four. The chromophore is stereochemically symmetrical but structurally unsymmetrical because of the presence of one cyclic and one acyclic terminal group. All six effective double bonds, *viz.*, *1,3,5,6,7*, and *9*, may give rise to different mono-*cis*-isomers.

This set has not yet been investigated in detail. The observed main fraction, "neo-γ-carotene", has a spectral difference of 5 mμ and must contain mainly

a mono-*cis* compound. Pro-γ-carotene is probably *3,5,7,9,11*-penta-*cis*-γ-carotene.

4. Stereochemical sets with an even number of conjugated carbon–carbon double bonds

(a) α-Carotene set (figures 3, 26, 27) (120)

The number of possible stereoisomers is thirty-two. The chromophore is structurally unsymmetrical because of the different positions of the double

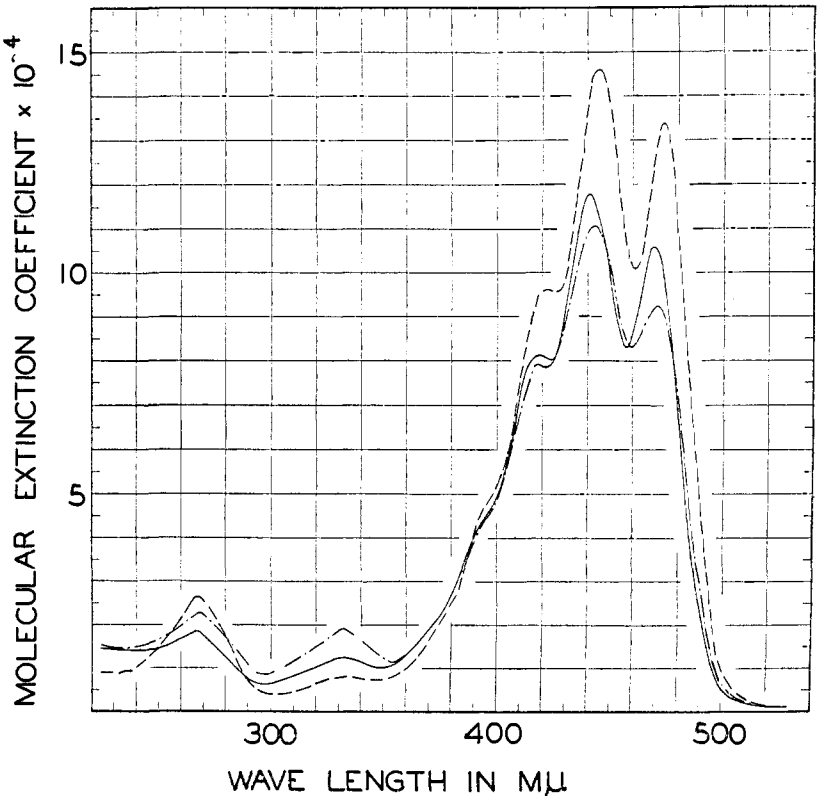


FIG. 26. Molecular extinction curves of neo-α-carotene U, compared with that of all-*trans*-α-carotene, in hexane: — — —, all-*trans*-α-carotene; ——, fresh solution of neo-α-carotene U; —·—, mixture of stereoisomers obtained from neo U upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 142 (1944))

bonds in the rings and also stereochemically unsymmetrical because of the even number of conjugated double bonds. That the two "central" double bonds, *5* and *6*, are structurally different follows from the position of the methyl side chains. The effective double bonds are *3,5,6,7*, and *9*. Considering these features, five mono-*cis*, ten di-*cis*, ten tri-*cis*, five tetra-*cis*, and one penta-*cis* members of the α-carotene set can be expected (*cf.* p. 280).

The neo-α-carotenes U, V, and W show considerable thermostability and very moderate *cis*-peaks. The isomers U and W seem to contain one *cis* double bond, whereas V must be reasonably interpreted as a di-*cis* compound. The most probable configurations are: neo U = *9*-mono-*cis*-α-carotene; neo W = *3*-mono-*cis*-α-carotene; and neo V = *3,9*-di-*cis*-α-carotene. A di-*cis* compound, neo X, was preponderantly formed upon mild heating of neo U solutions; therefore one of its *cis* double bonds, *viz. 9*, will probably have a position identical with that of neo U. Since the *5,9* and *6,9* configurations must be assigned to other isomers (see below), neo-α-carotene X is probably *7,9*-di-*cis*-α-carotene.

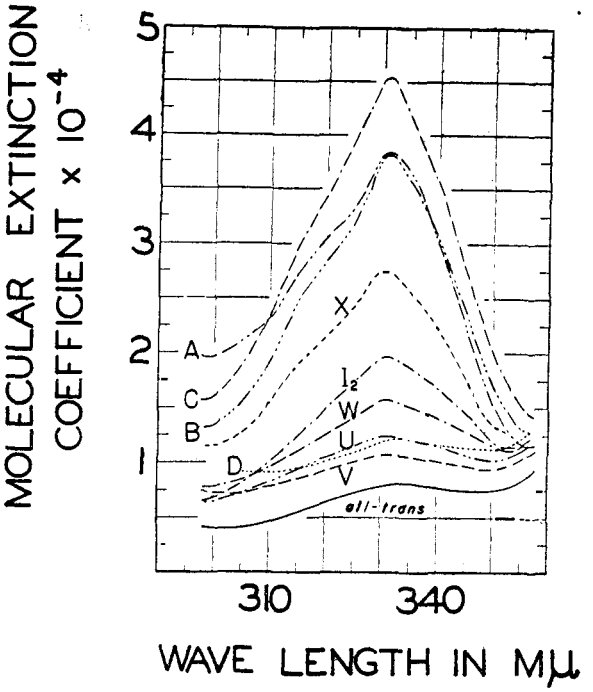


FIG. 27. Molecular extinction curves of some members of the stereoisomeric α-carotene set in the *cis*-peak region in hexane. I_2 indicates the equilibrium mixture obtained upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 140 (1944))

Neo C, a labile minor isomer, could be *6*- or *5*-mono-*cis*-α-carotene. One of the main observed isomers, neo B, has approximately the same *cis*-peak, and in the visible region the same spectral difference from the all-*trans* maximum as neo-β-carotene B. Its high peak suggests that one of its *cis* positions is central and the other peripheral, because neither two peripheral nor two central *cis* double bonds would explain the height of the peak observed. The non-equivalence of the *5*- and *6*-positions of the models shows that neo-α-carotene B may be either *5,9*- or *6,9*-di-*cis*-α-carotene. Since the neo forms A and B have practically identical peaks, it is reasonable to say that for these two di-*cis* isomers the following four configurations are possible: *5,9*; *6,9*; *3,5*; and *3,6*.

(b) Lutein set (figure 28) (120)

The characteristics of this dihydroxy-α-carotene set are identical with those of α-carotene. Only two main observed isomers, neo A and B, have been investigated. Neolutein A is very probably *6*- or *5*-mono-*cis*-lutein. The extinction at the *cis*-peak of neo B is less than the half of that of neo A. The difficulty in assigning a definite configuration to neo B lies in the fact that the visual spectra of A and B differ only by 1 mμ. Neolutein B could be either *3*-mono-*cis*-or *9*-mono-*cis*-lutein.

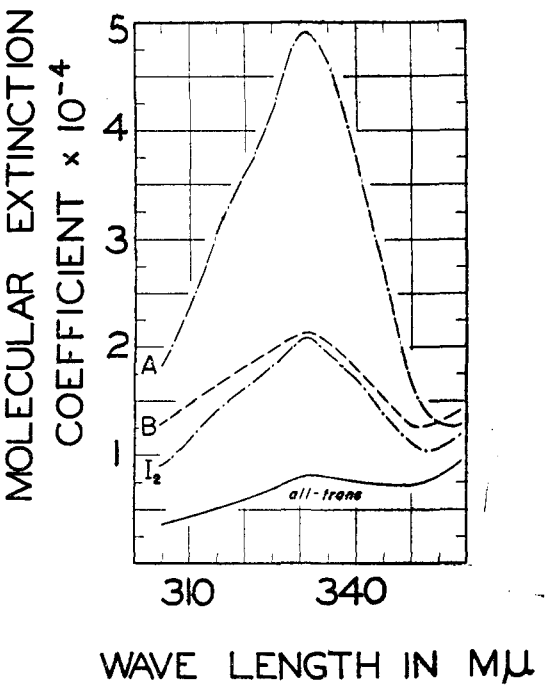


FIG. 28. Molecular extinction curves of some members of the stereoisomeric lutein set in the *cis*-peak region in hexane. I_2 indicates the equilibrium mixture obtained upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 140 (1944))

(c) Capsanthin set (figure 29) (67)

The number of possible stereoisomers is thirty-two. This set contains ten conjugated carbon–carbon double bonds in conjugation with one carbonyl group. The chromophore is structurally and stereochemically unsymmetrical. The five effective double bonds are *3*,*5*,*6*,*7*, and *9*.

The two main isomers, neo A and B, are evidently mono-*cis* compounds, as shown by the identical position of their visually observed maxima. On the basis of the high *cis*-peak of A and the considerable peak of B the most probable configurations are: neocapsanthin A = *6*-mono-*cis*-capsanthin and neocapsanthin B = *5*-mono-*cis*- or *7*-mono-*cis*-capsanthin. The positions of the two *cis*

double bonds in the minor isomer neocapsanthin C cannot be stated definitely; the models do not exclude the possibility that one of its *cis* positions is at or near the center.

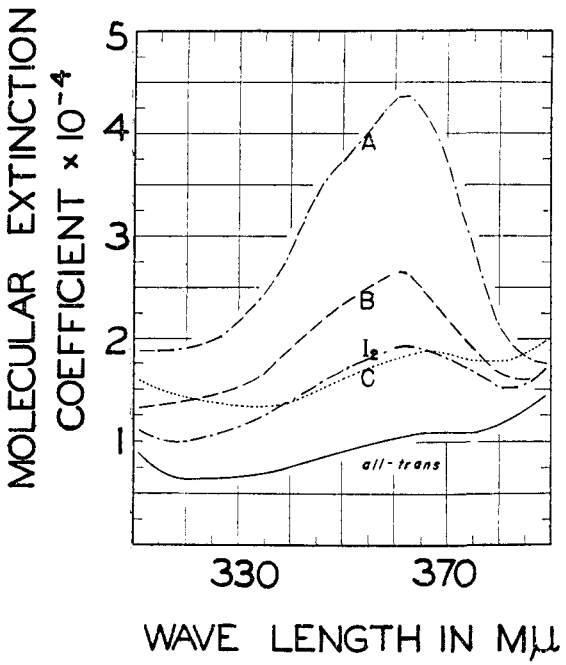
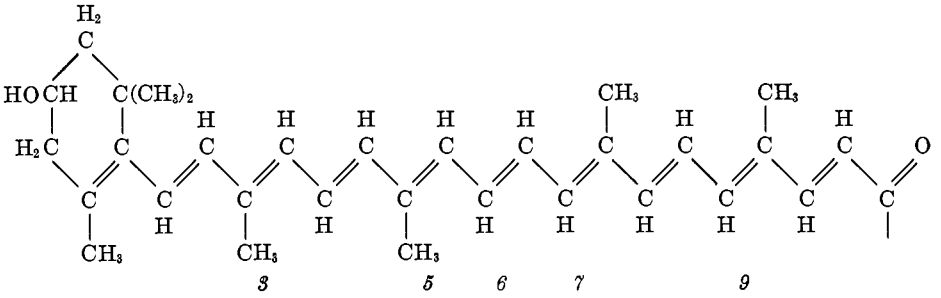


FIG. 29. Molecular extinction curves of some members of the stereoisomeric capsanthin set in the *cis*-peak region in benzene. I_2 indicates the equilibrium mixture obtained upon iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 189 (1944))



The chromophore of capsanthin
(The sterically effective double bonds are numbered)

III. CAROTENOIDS CONTAINING LESS THAN FORTY CARBON ATOMS

Although the quantity of the natural pigments belonging to this class is negligible compared with the amounts of C_{40}-carotenoids which are synthesized in the vegetable tissue each year, these pigments are, nevertheless, important from

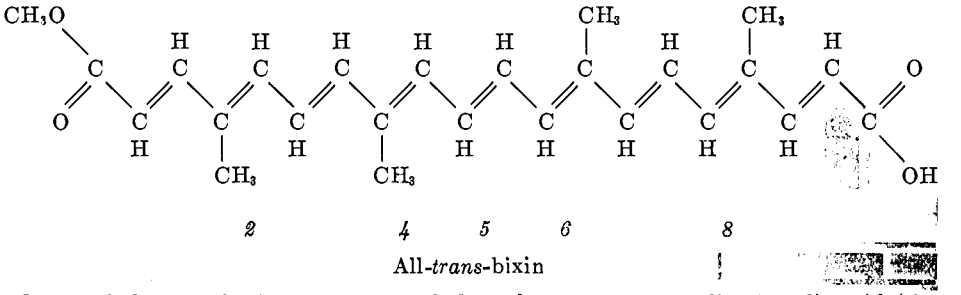
the stereochemical viewpoint. Indeed, some of the fundamental observations in this field were made with bixin and crocetin at a time when nothing was known of stereoisomeric polyenes with forty carbon atoms.

Bixin, $C_{25}H_{30}O_4$, occurs in the seed hulls of the tropical Annatto tree, *Bixa orellana L.*, and constitutes the main pigment of the industrial annatto or Orlean. A pigment of still lower molecular weight, crocetin, $C_{20}H_{24}O_4$, is present in the stigma of saffron (*Crocus sativus*) in the form of the gentiobioside, crocin. Certain other flowers also contain crocetin.

The formulas show the presence of nine and seven conjugated carbon–carbon double bonds in conjugation with carboxyl groups. As can be seen from the characteristic position of the methyl side chains, the chromophores are identical with the middle section of the C_{40}-carotenoids. The correctness of the symmetrical formulas was proved by Karrer *et al.* (21).

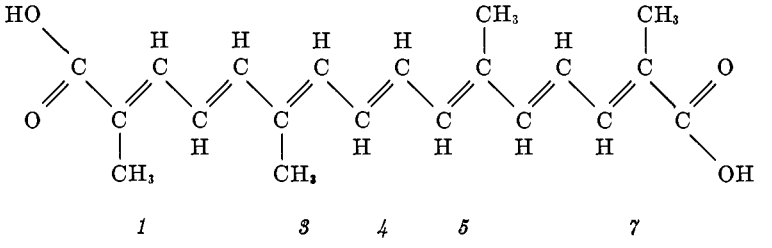
The bixin molecule contains five stereochemically effective double bonds. Considering its non-symmetrical structure, the calculated number of stereoisomers is thirty-two (as for α-carotene), whereas in the case of the free carboxylic acid, norbixin, or its dimethyl ester, methylbixin, this number is reduced to twenty (as for β-carotene).

Crocetin constitutes a similar case with five effective double bonds. The number of possible stereoisomers is twenty for crocetin or dimethylcrocetin but thirty-two for methylcrocetin.



All-*trans*-bixin

Monomethyl ester of 3,7,12,16-tetramethyloctadecanonaene-1,18-dicarboxylic acid (the stereochemically effective double bonds are numbered in italics); the dimethyl ester is termed methylbixin, and the free dicarboxylic acid, norbixin



All-*trans*-crocetin

1,5,10,14-Tetramethyl-tetradecaheptaene-1,14-dicarboxylic acid, (the stereochemically effective double bonds are numbered in italics); the methyl esters are termed methylcrocetin and dimethylcrocetin

A. BIXIN

The history of bixin stereochemistry reaches as far back as 1923, when Herzig and Faltis (17), in a single, unreproducible experiment, obtained from Orlean, instead of the well-known bixin ("labile bixin"), an isomer, "β-bixin" ("stable bixin"), with higher melting point, greater stability, and longer wave-length spectrum. The same starting material gave ordinary bixin in other experiments. An analogous observation was reported later by Karrer, Helfenstein, Widmer, and van Itallie (21). These investigators were the first to express the opinion that the two bixins are in a *cis–trans* relationship (*cf.* 31), a fact which was proved by the conversion of ordinary bixin into the β-bixin by means of iodine. If iodine is added to an ethyl acetate solution of methylbixin, the methyl ester of the β-bixin crystallizes out on short standing. According to Kuhn and Winterstein (38), catalytic amounts of the halogen are sufficient to bring about this conversion. Perbenzoic acid in chloroform is also effective (88).

The structural identity of the two bixins, in spite of spectroscopic differences (table 22), was proved chemically by Kuhn and Winterstein (38) and Kuhn and

TABLE 22

Visually observed spectral maxima of some members of the methylbixin set, listed in the sequence of decreasing adsorption affinities

MEMBER OF THE SET	IN PETROLEUM ETHER (B.P. 60-70°C.)		IN BENZENE	
	mμ	mμ	mμ	mμ
Natural methylbixin	485	453.5	503	470
All-*trans*-methylbixin	490	457	508.5	475
Neomethylbixin A	485	454	502.5	469
Neomethylbixin B	471	444.5	491	458
Neomethylbixin C	479.5	449	496	463

Drumm (29, 30). When two hydrogen atoms were added to either bixin by treatment with zinc and glacial acetic acid or with titanium trichloride and ammonia (88), the same dihydro product appeared in both experiments. This is geometrically understandable in the case of a Thiele addition of two hydrogen atoms to *cis–trans* isomers. By oxidation in air, in the presence of pyridine, dihydrobixin formed that bixin which can be obtained from natural bixin by iodine catalysis. The total transition is:

$$\text{natural bixin} \rightarrow \text{dihydrobixin} \rightarrow \beta\text{-bixin}$$

In the following cases a chemical change produced a direct transition from "labile" into "stable" compound: labile methylbixin when saponified with aqueous-alcoholic alkali gave stable norbixin; alkali converted labile methylbixin (to a small extent) into stable bixin; and labile norbixin when methylated with methanolic hydrogen chloride gave stable methylbixin instead of the expected labile form, which can be obtained by the action of diazomethane or dimethyl sulfate on norbixin (for details see 102). An "isobixin" which had been

described by van Hasselt (14) could not be reproduced by Karrer and Takahashi (23); its homogeneity is doubtful.

When all these observations are considered, natural bixin must contain at least one *cis* double bond in a position where the artifact is *trans*. This also causes a difference in adsorbability, as pointed out in a brief remark of Winterstein and Stein (99).

The nomenclature which has been employed so far includes the following designations for the two bixins and analogous terms for the two methylbixins and norbixins:

(*a*) Ordinary bixin = natural bixin = *cis*-bixin = α-bixin = labile bixin = bixin II = lower melting bixin (m.p. 198°C. (corr.))

(*b*) Isobixin = *trans*-bixin = β-bixin = stable bixin = bixin I = higher melting bixin (m.p. 220°C. (corr.))

"Stable" bixin is undoubtedly the all-*trans* member of the set.

Neither the number nor the position of the *cis* double bonds in the naturally occurring "labile" pigment had been determined in the investigations discussed above. So far as we know, the only approach to a solution of this problem was made by Karrer and Solmssen (22), who cautiously oxidized the two bixins with permanganate. In both cases the aldehydes I, II, and III, which evidently arose through a stepwise splitting of the chain near the free carboxyl group in bixin were observed:

(I) $\cdots{=}\mathrm{C}(\mathrm{CH_3})\text{—}\mathrm{CH}{=}\mathrm{CH}\text{—}\mathrm{CH}{=}\mathrm{C}(\mathrm{CH_3})\text{—}\mathrm{CHO}$ (6, 7, 8)

(II) $\cdots{=}\mathrm{C}(\mathrm{CH_3})\text{—}\mathrm{CH}{=}\mathrm{CH}\text{—}\mathrm{CHO}$ (6, 7)

(III) $\cdots{=}\mathrm{C}(\mathrm{CH_3})\text{—}\mathrm{CHO}$ (6)

The aldehydes I and II, when prepared by oxidation of stable bixin, were the *trans* forms of those obtained from labile bixin, but an identical all-*trans* aldehyde III resulted from both bixins. The authors, therefore, concluded (with reservation) that the double bond 7 has *cis* configuration in natural bixin. Although this conclusion must now be revised because bond 7 is a sterically hindered double bond, the remarkable method reported by Karrer and Solmssen may become useful in the future.

A recent re-investigation of the sterically simpler methylbixin set, in collaboration with Escue (111), gave the following results:

After isomerization and development with benzene–petroleum ether mixtures on calcium carbonate columns, in addition to the two well-known methylbixin isomers, two others, neo A and C, were isolated in crystalline form. Some characteristics of a fifth minor isomer, neo B, were determined in solution and a few other isomers occurring in traces were observed occasionally. Since "labile" methylbixin is much less labile than some other members of the set, it has been renamed "natural methylbixin", a name which implies that it has the same

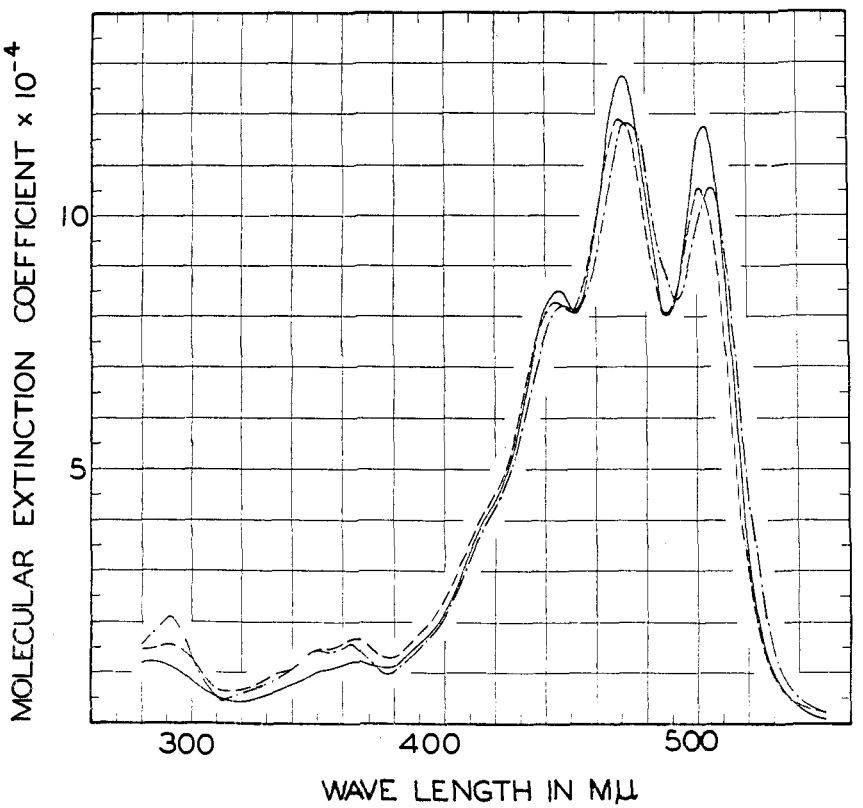


FIG. 30. Molecular extinction curves of natural methylbixin in benzene: ——, fresh solution of natural methylbixin; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 323 (1944))

configuration as the bixin occurring in the *Bixa* seeds. Stable or *trans*-methylbixin is now termed "all-*trans*-methylbixin." Characteristic spectral data are given in table 22, in which the isomers are listed in the sequence of decreasing adsorption affinity. Upon addition of iodine the isomers shift their maxima to the following wave lengths; in petroleum ether, 488, 455 mμ; and in benzene, 506.5, 472 mμ.

The extent of spontaneous isomerization of natural methylbixin in benzene

at room temperature is about 3 per cent within 24 hr. After refluxing each pure isomer for 1 hr. the extent of stereoisomerization was: natural methylbixin, 26 per cent (mainly neo C formed); all-*trans*-methylbixin, 37 per cent (mainly neo C); neomethylbixin A, 68 per cent (mainly all-*trans*); and neomethylbixin C, 54 per cent (mainly natural methylbixin formed).

Extensive *cis–trans* rotations occur also when the crystals are melted and kept at 160°C. for 1 min. The composition of the iodine equilibrium mixture

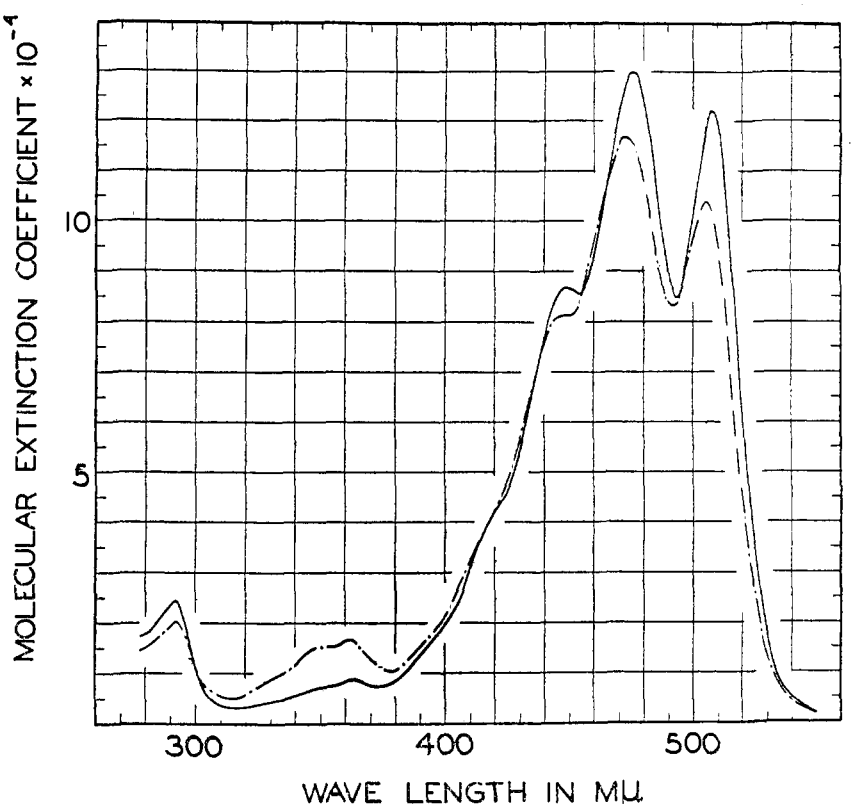


FIG. 31. Molecular extinction curves of all-*trans*-methylbixin in benzene: ——, fresh solution of the all-*trans* compound; —·—, after iodine catalysis at room temperature in light. The second curve is practically identical with that obtained after refluxing for 45 min. in darkness. (From J. Am. Chem. Soc. **66**, 323 (1944))

is (with some variations): all-*trans*:neo A:neo C = 68:22:10. The configuration of natural methylbixin is so sensitive to iodine that this isomer practically disappears when the catalyst is added to its solution.

Extinction curves for bixins have been published by Smakula and by Karrer and Würgler (23a, 73). In figures 30 to 33 we give such curves for the four crystalline isomers, as well as for the stereoisomeric mixtures obtained upon refluxing or iodine catalysis. The changes observed upon such treatment are analogous

to those which take place with C_{40}-carotenoids. As illustrated by figure 34, light is needed for the development of the *cis*-peak in the presence of iodine. The *cis*-peak (like that of lycopene) possesses a characteristic fine structure, the cause of which is unknown.

With the exception of the all-*trans* form, the isomers studied are light sensitive, especially the neomethylbixins A and C. Under the conditions applied, no

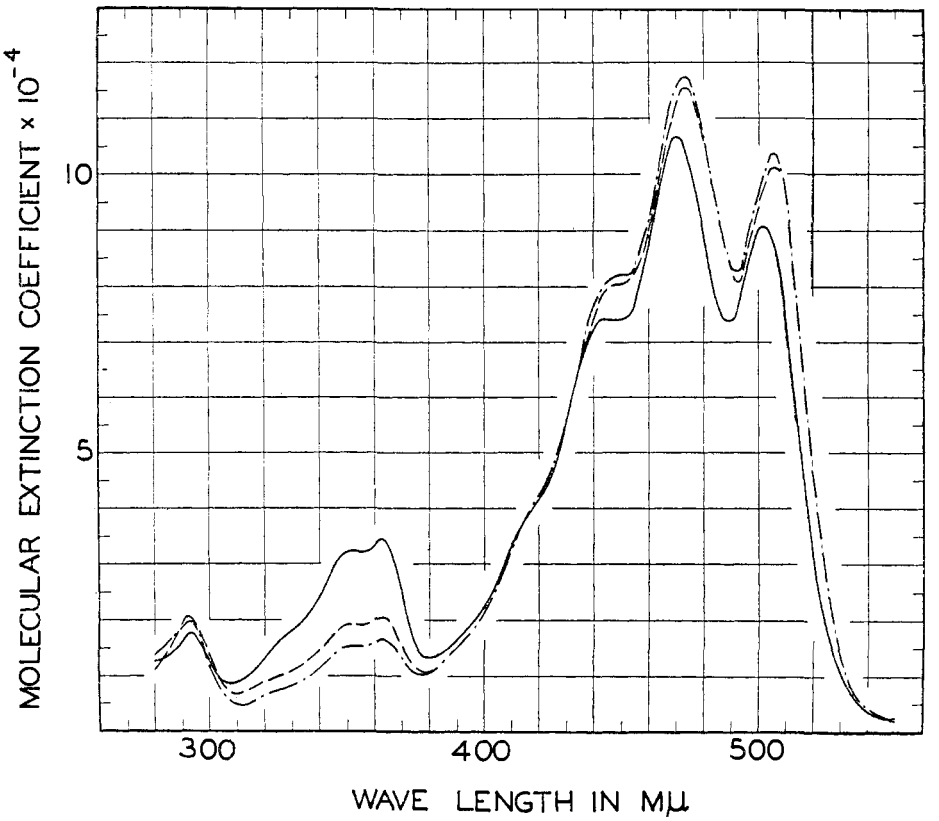


FIG. 32. Molecular extinction curves of neomethylbixin A in benzene: ——, fresh solution of neomethylbixin A; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 324 (1944))

irreversible conversion or bleaching occurred during the exposure to sunshine, the effect of which is demonstrated by figures 35 and 36 (p. 330).

Assignment of configurations

Let us consider now the configurations. On the basis of the spectral differences from the all-*trans* compound in the visual region (table 22), natural methylbixin and neo A are doubtlessly mono-*cis* compounds but neo C is a di-*cis*- and B a tri-*cis*(or possibly tetra-*cis*)-methylbixin. It is a peculiar feature of this set that upon refluxing or insolation of natural methylbixin practically no all-*trans*

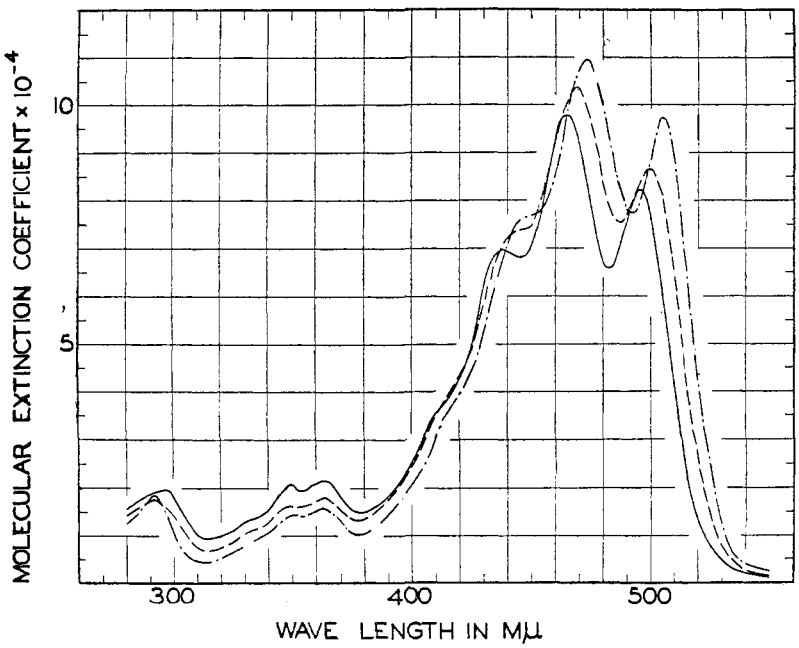


FIG. 33. Molecular extinction curves of neomethylbixin C in benzene: ——, fresh solution of neomethylbixin C; — — —, mixture of stereoisomers after refluxing in darkness for 45 min.; —·—, mixture of stereoisomers after iodine catalysis at room temperature in light. (From J. Am. Chem. Soc. **66**, 325 (1944))

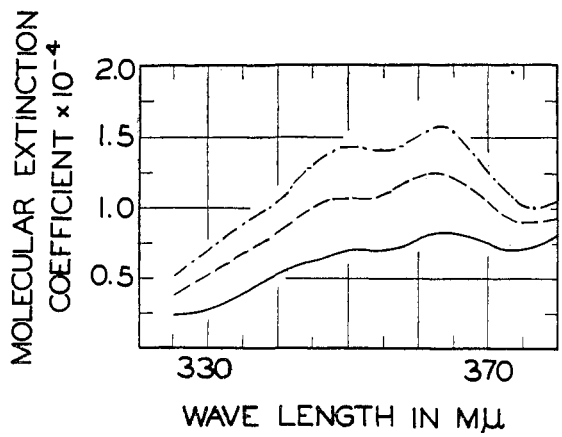


FIG. 34. Influence of illumination on the development of the *cis*-peak in an iodine-catalyzed solution of all-*trans*-methylbixin in benzene: ——, molecular extinction curve after standing in darkness with iodine for 30 min.; — — —, molecular extinction curve after 5 sec. illumination; —·—, molecular extinction curve after 30 sec. illumination. (From J. Am. Chem. Soc. **66**, 325 (1944))

form appears, and *vice versa*. In the absence of catalysts the stability of the *cis* double bond in natural methylbixin is so great that the molecule undergoes a

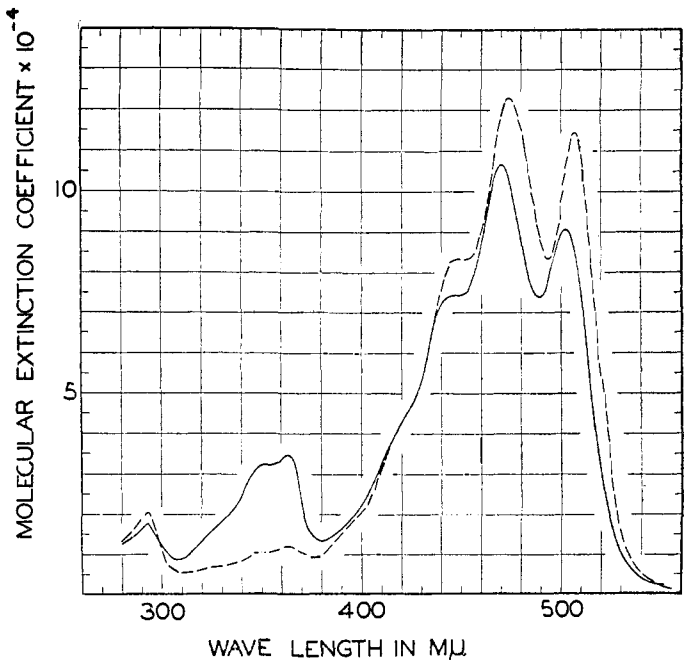


FIG. 35. Photo-isomerization of neomethylbixin A in benzene: ——, fresh solution of neomethylbixin A; — — —, mixture of stereoisomers after insolation for 15 min. (From J. Am. Chem. Soc. **66**, 326 (1944))

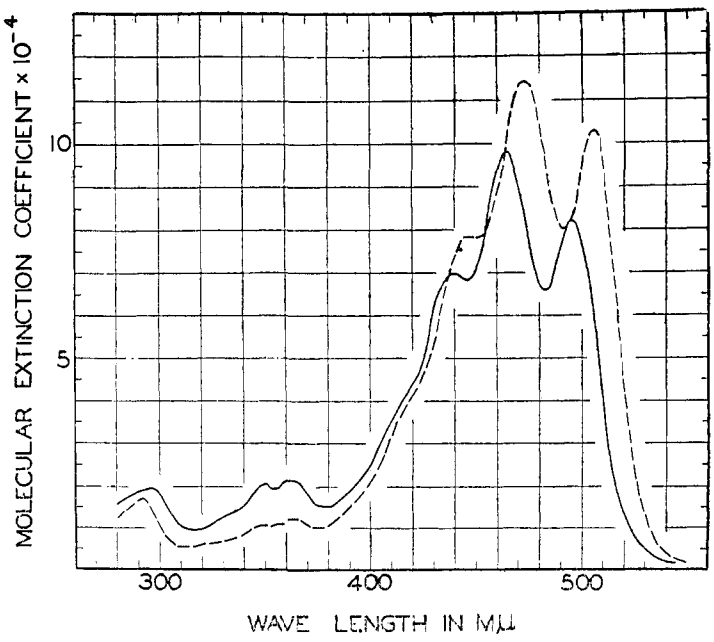


FIG. 36. Photo-isomerization of neomethylbixin C in benzene: ——, fresh solution of neomethylbixin C; — — —, mixture of stereoisomers after insolation for 15 min. (From J. Am. Chem. Soc. **66**, 327 (1944))

second *trans* → *cis* rotation and gives neo C rather than yield marked amounts of the all-*trans* compound. On the other hand, all-*trans*-methylbixin is easily converted into neo A and *vice versa*. These isomerizations obviously affect another double bond than that which is *cis* in natural methylbixin. Therefore, we have the following scheme, in which the dotted arrows indicate hindered

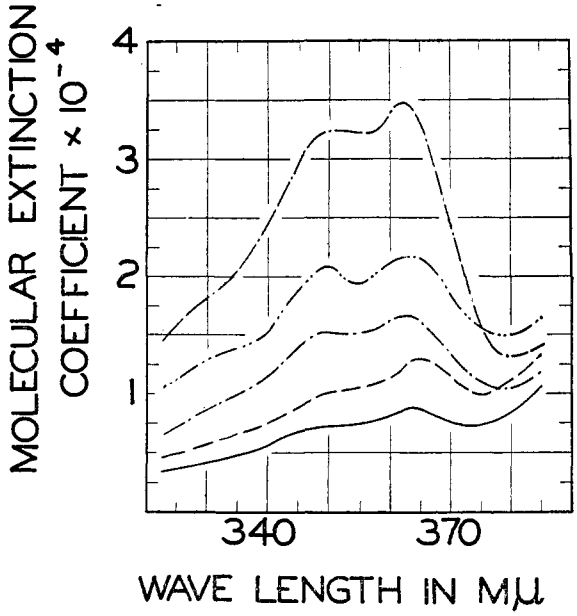
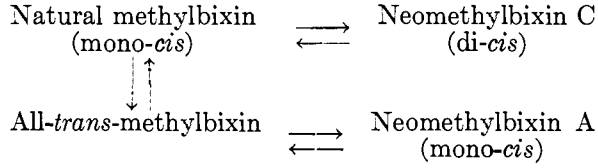


FIG. 37. Molecular extinction curves of some members of the stereoisomeric methylbixin set in the *cis*-peak region in benzene: ——, all-*trans*-methylbixin; — — —, natural methylbixin; —·—, iodine equilibrium mixture; —··—, neomethylbixin C; — — ·— —, neomethylbixin A. (From J. Am. Chem. Soc. **66**, 327 (1944))

interconversion and the full arrows easy interconversions, if the pigment is refluxed or insolated.



The molecular extinction coefficients × 10^{-4} at the *cis*-peak wave length (figure 37), minus the corresponding value for the all-*trans* compound, are as follows:

Natural methylbixin	0.4	Neomethylbixin A	2.8
All-*trans*-methylbixin	0	Neomethylbixin C	1.4

For neo B, because of its lability, it can be only claimed qualitatively that its extinction curve is flat in the *cis*-peak region.

On the basis of the spectral data, the mono-*cis* compound A, because of its exceptionally high *cis*-peak, must be assigned the configuration of *5*-mono-*cis*-methylbixin. Natural methylbixin, which possesses an increased adsorption affinity and a low *cis*-peak, must have (in analogy to neo-β-carotene U) a peripherally located *cis* double bond and is *2*-mono-*cis*-methylbixin. On the basis

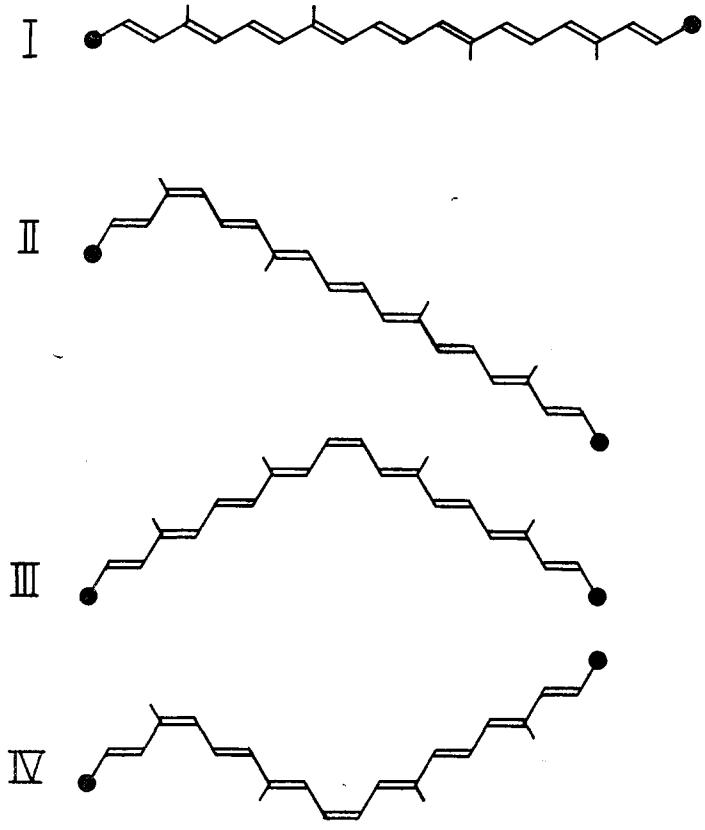


FIG. 38. Suggested stereochemical structures of the four crystalline members of the methylbixin set: I, all-*trans*-methylbixin; II, natural methylbixin; III, neomethylbixin A; IV, neomethylbixin C. The carboxyl groups are represented by black circles. (From J. Am. Chem. Soc. **66,** 327 (1944))

of the scheme given on p. 331, it can be claimed that neo C is probably *2,5*-di-*cis*-methylbixin. The skeleton models of these isomers are given in figure 38.

The above assignment of configurations is in reasonable agreement with the ratio of the observed principal absorption peaks in the visible region. For the four main members of the methylbixin set the experimentally found maximum intensity ratios are all-*trans*: natural: neo A:neo C = 1:0.93:0.80:0.82, while the ratios calculated according to Pauling (62) on the basis of the models (with the single-bond–double-bond angles taken as 125° 16′) are 1:0.87:0.82:0.79.

This agreement is satisfactory, considering the experimental error and the approximate nature of the theory.

Our configurational assignments (111) are also in accordance with some earlier data of the literature. Already van Hasselt (13, 14) has shown that the molecule of naturally occurring bixin possesses two non-equivalent terminal groups because it forms two different methyl ethyl esters. This feature was first explained by a structurally non-symmetrical bixin formula. Karrer, Helfenstein, Widmer, and van Itallie (21), however, correctly pointed out that the cause is stereochemical. Consequently, the *cis* double bond cannot have a central location in natural methylbixin and one of three possible positions appears to be excluded by a purely chemical argument.

B. CROCETIN

The first observation of stereoisomeric crocetins was made by Kuhn and Winterstein (39, 40) in 1933. They isolated dimethylcrocetin by extraction and exchange esterification of the natural crocetin gentiobioside (crocin) with methyl alcohol and obtained a second dimethylcrocetin with much lower melting point (141°C.), in addition to the well-known compound (m.p. 220°C.). The yield of the new isomer was 1 g. from 1 kg. of dry saffron, while the ordinary dimethylcrocetin can be isolated in quantities of 17 to 60 g. This result shows that two stereoisomeric crocins must be present in the stigma. Unfortunately, it came to light later (32, 33) that the specimen of saffron used in the experiments mentioned was rather exceptional, and that most samples contain only the higher-melting isomer.

The biologically important occurrence of both *cis*- and *trans*-dimethylcrocetins in some algae, where they are active as sex-stimulating and directing agents, can only be mentioned; some fundamental observations were made by Kuhn, Moewus, *et al.* (27, 32, 33, 34, 35).

Because there is no generally accepted nomenclature in this field, the following terms have been used:

(*a*) Ordinary crocetin = *trans*-crocetin = stable crocetin = crocetin I

(*b*) *Cis*-crocetin = labile crocetin = crocetin II (known in form of derivatives)

The monomethyl and dimethyl esters of (*a*) were earlier termed β-crocetin and γ-crocetin, respectively, while the free dicarboxylic acid itself was called α-crocetin. This nomenclature is now obsolete.

The isomer (*a*) is certainly the all-*trans* compound; nothing seems to be known about the position or even the number of *cis* double bonds in (*b*). The spectral differences indicate one such bond (table 23, p. 334).

According to Kuhn and Winterstein (39), the labile dimethylcrocetin has a great tendency to rearrange and thus to form the stable isomer. This conversion can be achieved by the following methods: If the *cis* pigment is heated above its melting point (141°C.) and cooled, crystals of the *trans* form appear. These crystals possess a higher melting point (220–1°C.) than the starting material.

If a concentrated petroleum ether solution of the *cis* form is heated in the presence of a trace of iodine, crystals of *trans*-dimethylcrocetin are deposited within a short time.

The same *cis* → *trans* isomerization can be achieved very easily also by irradiation. Indeed, even during measurements in the visual spectroscope, the bands migrate toward longer wave lengths until they reach approximately those positions characteristic of the stable form. The spectral region between 400 and 500 mμ is the most effective, while ultraviolet or red light does not cause stereoisomerization. Irradiation of a petroleum ether solution with a Nitra-Osram lamp (9 volts, 3.9 amperes) at a distance of 35 cm., shifted the first spectral band from 446 mμ to 450.5 mμ within 15 min., by which time the process had been completed. The photosensitivity in a number of other solvents is similar.

If labile dimethylcrocetin is boiled with zinc dust in pyridine in the presence of some glacial acetic acid, the color changes from orange-yellow to sulfur-yellow. Upon the addition of sodium hydroxide, the liquid becomes dark greenish blue,

TABLE 23

Comparison of "stable" (all-trans) and "labile" (mono-cis) dimethylcrocetins (39)

PHYSICAL PROPERTIES	STABLE FORM	LABILE FORM
Crystal form	Hexagonal plates	Long, rectangular
Color under the microscope	Reddish orange	Yellow
Melting point (corrected), °C.	222	141
Solubility in methanol at 20°C.	1:100,000	1:6,000
Solubility in ether	Lower	Higher
Spectral maxima in petroleum ether (b.p. 70–80°C.)	450.5 424.5 mμ	445 422 mμ
Spectral maxima in chloroform	463 434.5 mμ	458 432.5 mμ

and shaking in air instantaneously produces the orange color of the stable form.

Thus the following chemical transformation which is analogous to that in the bixin set, has been achieved:

"labile" dimethylcrocetin → dihydrodimethylcrocetin → "stable" dimethylcrocetin

We are able to predict that the reported stereoisomeric changes will be found to be reversible, and furthermore that several new members of the set can be observed and isolated. Chromatographic separation should offer no difficulty, and has indeed been claimed for the two dimethylcrocetins by Winterstein and Stein (99) as well as by Winterstein (98) but, unfortunately, experimental directions are lacking.

C. AZAFRIN

As pointed out by Kuhn and Winterstein (39), azafrin, $(HO)_2C_{26}H_{35}COOH$, which occurs in the Azafranillo root (*Escobedia scabrifolia, E. linearis*, Schl.)

(28, 42) very probably possesses an all-*trans* configuration. Its methyl ester was regenerated unchanged in melting point and spectrum from an iodine addition product; the spectral bands of the natural pigment did not shift their position when illuminated.

IV. Diphenylpolyenes

A. General remarks

In the series of the diphenylpolyenes, $C_6H_5(CH{=}CH)_nC_6H_5$, some stereochemical aspects of the simplest representatives, stilbene ($n = 1$) and diphenylbutadiene ($n = 2$), were the subjects of early investigations, but until recently the data available referred only to these compounds.

The methods of synthesis for higher members, up to $n = 8$, were, to a large extent, developed by Kuhn and Winterstein (37; for $n = 15$ see 36a). They found that even when a higher diphenylpolyene was synthesized by ten different methods, the same compound was isolated in each instance. Evidently the conditions favor the formation of the most stable stereoisomer in each set, i.e., the "ordinary" diphenylpolyene. That this is the all-*trans* member of the set has been proved for diphenylhexatriene and diphenyloctatetraene by the x-ray studies of Hengstenberg and Kuhn (15).

According to Kuhn and Winterstein (37), in this unbranched and symmetrical series the number of possible stereoisomers, N, of a compound which contains n double bonds is

$$N = 2^{n-1} + 2^{p-1}$$

where $n = 2p$ for an even number and $n = 2p - 1$ for an odd number of aliphatic conjugated double bonds.

It has, however, recently been pointed out by LeRosen and the author (114, 115) that by no means all stereoisomers may be formed with equal probability. All possible members of a given stereoisomeric set can be classified into two types: one type (*a*) possesses *cis* configuration about one or both double bonds adjacent to the benzene rings, while the other (*b*) shows *trans* configuration in these bonds.

The probability of the formation in any substantial quantity of isomers belonging to type (*a*) is much smaller because of the steric conflict represented in figure 39. This figure shows that a hydrogen atom of the ring (in ortho position to the side chain) is spatially hindered by one of the side-chain hydrogens, so that an approximately planar configuration becomes impossible. The deviation will be about 52.5°. Therefore, if an all-*trans* diphenylpolyene isomerizes, the formation of those members which possess *trans* configuration on both ends of the aliphatic system will be favored.

In the limiting case of *cis*-stilbene (47), hydrogen atoms attached to different rings interfere with each other (figure 40). The difference in optical properties of *cis*- and *trans*-stilbenes has been interpreted by Lewis and Calvin (46) on the assumption that the repulsion of the two ortho hydrogen atoms forces the mole-

cule out of a plane and diminishes resonance. On a similar basis the energy difference between the two stereoisomers (10 kcal.) can be explained (Lewis, Magel, and Lipkin (47)).

In the stilbene and diphenylbutadiene sets only sterically hindered stereoisomers are possible; in higher diphenylpolyenes, however, the *trans* → *cis* isomerization of the all-*trans* form will be distributed between both types. The isomers which can be formed without significant steric hindrance will, however, be preferred and their formation can therefore be expected in substantially larger quantities than those which involve a definite spatial conflict. This

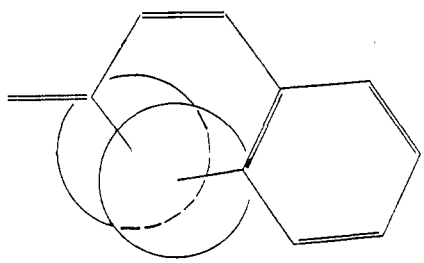


FIG. 39. Model of one molecule end of diphenyloctatetraene. Values used: C=C, 1.33 Å.; C—C, 1.46 Å.; C_6H_5—C, 1.44 Å.; C_6H_5—H, 1.08 Å.; C—H, 1.09 Å.; H radius, 1.20 Å.; angles C=C—C and C=C—H, 124°20′. (From J. Am. Chem. Soc. **64**, 2757 (1942))

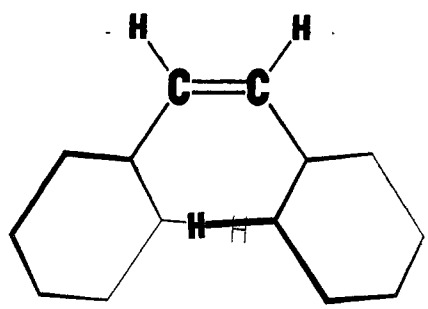


FIG. 40. Overlapping of hydrogen atoms in *cis*-stilbene (From J. Am. Chem. Soc. **62**, 2977 (1940))

prediction is in accordance with some observations in the diphenyloctatetraene set to be discussed below (p. 339).

The number of possible stereoisomers of each type is given for certain diphenylpolyenes in table 24.

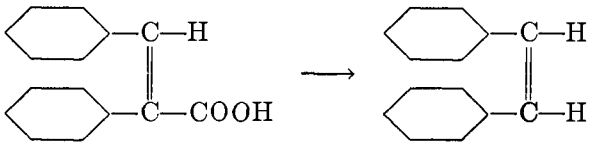
B. 1,2-DIPHENYLETHYLENE (STILBENE AND ISOSTILBENE), $C_6H_5CH{=}CHC_6H_5$

As is well known, the literature dealing with this simplest polyene is extensive, but the discussion of the following data may suffice.

Cis-stilbene, also termed "isostilbene", which was detected by Otto and

Stoffel (59) as early as in 1897, may be prepared either by photochemical or by purely chemical methods. Stoermer (79) obtained a small quantity of isostilbene from the *trans* form by prolonged irradiation with ultraviolet light, and a similar method was used recently by Lewis, Magel, and Lipkin (47). The quantum yields of such an irradiation were studied by Smakula (74). Stoermer and Prigge (80) early reported data concerning the effect of irradiation on some stilbene derivatives.

The older chemical method for the preparation of isostilbene, *viz.*, the partial reduction of diphenylacetylene (tolane), has now gone into discard in favor of the Stoermer method employing decarboxylation of α-phenylcinnamic acid:



α-Phenylcinnamic acid Isostilbene

TABLE 24

The two types of cis-diphenylpolyenes, $C_6H_5(CH{=}CH)_nC_6H_5$

STEREOISOMERIC SET	n	NUMBER OF STEREOISOMERS		
		Hindered	Not hindered	Total
Stilbene	1	1	0	1
Diphenylbutadiene	2	2	0	2
Diphenylhexatriene	3	4	1	5
Diphenyloctatetraene	4	7	2	9
Diphenyldecapentaene	5	14	5	19
Diphenyldodecahexaene	6	26	9	35

By the use of a copper chromite catalyst, Taylor and Crawford (89) have succeeded in improving this conversion so effectively that pure *cis*-stilbene can now be prepared in quantity, since the yields amount to 60–65 per cent.

The preparation of ring-substituted *cis*-stilbenes follows the same lines. For example, Ruggli and Staub (70) obtained nitro and amino derivatives; Späth and Kromp (77) prepared *cis*-pterostilbene by decarboxylation.

Although *cis*-stilbene had been described as an oil, Weygand and Rettberg (94) recently succeeded in obtaining a crystalline sample (m.p. 1°C.) by fractional precipitation with 1,3,5-trinitrobenzene, after a systematic elimination of such contaminants as tolane, dibenzyl, and *trans*-stilbene. Pure *cis*-stilbene is more thermostable than might be expected. Even at 214°C. only 8 per cent undergoes isomerization in 20 hr. (Taylor and Murray (90)). The kinetics of this process has been studied by Kistiakowsky and Smith (25).

In alcoholic solution *cis*-stilbene has its spectral maximum at 278 mμ and a molar extinction coefficient of 0.935×10^4. The maximum of the *trans* compound lies at a considerably longer wave length (294 mμ) and the molar extinc-

tion is 2.34×10^4. These data are comparable with those observed with the *cis*- and *trans*-cinnamic acids (5, 48, 75). For a new investigation in this field *cf.* Jones (19).

Cis-stilbene is easily isomerized by iodine (a process which is promoted by sunlight) and by other catalysts, for example, boron trifluoride (Price and Meister (68)). Kharasch, Mansfield, and Mayo (24) observed, however, that it is not isomerized by hydrobromic acid in the dark, either in the presence or in the absence of air. In contrast, the addition of benzoyl peroxide, ascaridole, or other peroxidic compounds to this system caused a rapid *cis* → *trans* change. Although *cis*-stilbene solutions are isomerized by hydrobromic acid in light, the process is less rapid in the absence of air. Because antioxidants, e.g., hydroquinone, hinder this *cis–trans* rotation, it is assumed that bromine atoms, liberated from the acid by light, oxygen, or a peroxide, are responsible for the process. The ineffectiveness of hydrochloric acid may be explained by its relatively high stability toward oxygen. A thorough discussion of related phenomena has appeared in this journal (Mayo and Walling (51); see also 90a).

For the practical separation of *cis*-stilbene and *trans*-stilbene use has been made of fractional distillation and crystallization, and recently of molecular addition products (Weygand and Siebenmark (95)). Spectrophotometric measurements, melting-point curves, and other methods (25, 47, 60, 68, 90, 94, 95, 95a) may be employed for the determination of the *cis* and *trans* components in mixtures. For the formation of mixed crystals see reference 93a.

It was reported recently by McNeely and the author (118) that a convenient procedure for the detection, purification, separation, and estimation of stereoisomeric stilbenes is to be found in the so-called chromatographic brush method (108, 112). After extrusion of the Tswett column containing an invisible chromatogram, a narrow streak is made down the cylinder with a brush which has been dipped into a 1 per cent permanganate solution. Where the reagent crosses a zone containing *cis* or *trans*-stilbene, it turns brown almost instantaneously. The zones thus located can be cut out and the isomers eluted after the streak has been shaved off. The application of this method to stilbene, *p*-methylstilbene, and *p*-methoxystilbene showed that in each case the *trans* form possesses the stronger adsorption affinity and is located near the top of the alumina column. Following this procedure, samples of *cis*- or *trans*-stilbene can be chromatographically tested for possible contamination by the other isomer. In a small-scale experiment the limit of detection is 1 to 2 per cent.

c. 1,4-DIPHENYLBUTADIENE, $C_6H_5(CH{=}CH)_2C_6H_5$

The three expected steric forms of this compound, *trans–trans*, *trans–cis*, and *cis–cis*, have been obtained by Straus (87) in the following way: When diphenyldiacetylene, $C_6H_5C{\equiv}C{-}C{\equiv}CC_6H_5$, is boiled with copper-coated zinc dust, a labile isomer of diphenylbutadiene is formed, which on long standing (or more rapidly in sunlight) can be converted into ordinary all-*trans*-diphenylbutadiene. In order to prepare the third member of the set, the reduction of diphenyldiacetylene was interrupted prematurely. The dihydro derivative

formed, $C_6H_5C{\equiv}C{-}CH{=}CHC_6H_5$, was further reduced and yielded a new labile diphenylbutadiene distinctly different from that just mentioned. Since this oily isomer is the most labile of the set and is easily converted by insolation into the crystalline all-*trans* form within 15 min., it may be reasonably assumed that it is the *cis–cis* form. The *trans–cis* configuration should be assigned to the isomer obtained by the copper–zinc method.

D. 1,6-DIPHENYLHEXATRIENE, $C_6H_5(CH{=}CH)_3C_6H_5$

No record of successful stereoisomerization of the triene seems to be available. However, the methods described below for the tetraene will very probably be satisfactory.

E. 1,8-DIPHENYLOCTATETRAENE, $C_6H_5(CH{=}CH)_4C_6H_5$

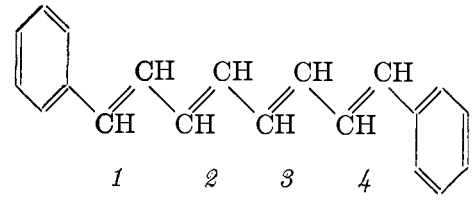
Until recently only the ordinary, all-*trans* form of this polyene was known. An alleged "white modification", observed earlier (78) and occasionally still mentioned in the literature (7), has been identified by Kuhn and Winterstein (37) as stilbene.

The stereochemical homogeneity of tetraene samples prepared by the customary methods of synthesis (37) can be demonstrated chromatographically; a single, fluorescing zone appears on the lime column. It was found in collaboration with LeRosen (114, 115), that it is easy to stereoisomerize all-*trans*-diphenyloctatetraene by some methods described in Part II. The mixture of stereoisomers obtained can be separated chromatographically on calcium hydroxide. After a few hours' refluxing in benzene and after development with petroleum ether containing 10 per cent of benzene, three fluorescing zones became visible when the column was illuminated with ultraviolet light. The weight ratio of the isomers, from top to bottom, was 83:15:2, i.e., about one-sixth of the starting material had undergone a spatial change. The top zone contains unchanged all-*trans* compound, while in the two other zones the two "unhindered" members of the set seem to be present (*cf.* table 24). It is reasonable to assume the following configurations (from top to bottom) (see the formula on p. 340):

trans-trans-trans-trans = all-*trans*-diphenyloctatetraene
trans-cis-trans-trans = *2-cis*-diphenyloctatetraene
trans-cis-cis-trans = *2,3*-di-*cis*-diphenyloctatetraene

In favorable cases two minor zones appeared below these main stereoisomers and very probably contained some of the spatially "hindered" members of the set.

Similar chromatograms can be obtained by treating the benzene solution of diphenyloctatetraene with some iodine for 15 min. or by keeping the melted crystals at 260°C. for the same time. Irradiation of solutions for 12 hr. with a mercury quartz lamp yielded, in addition to the main isomers, two minor members with markedly weaker adsorption affinity.



All-*trans*-diphenyloctatetraene
(The sterically effective double bonds are numbered; *1* and *4* are of the hindered type)

The bending of the tetraene molecule can also be demonstrated by means of the *cis*-peak effect. Upon addition of iodine to the benzene solution of the all

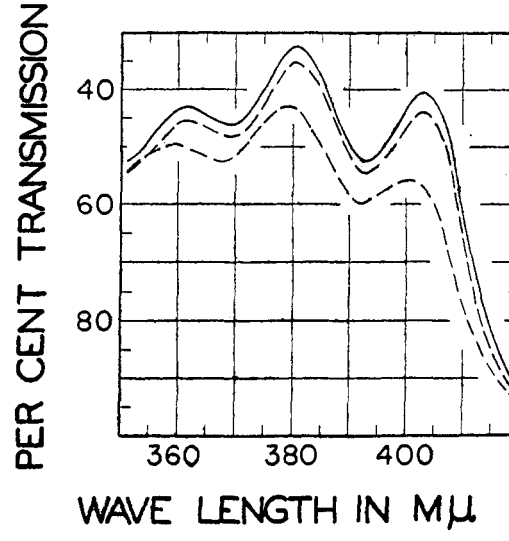


FIG. 41. Spontaneous re-isomerization of 2-*cis*-diphenyloctatetraene (and minor isomers) in benzene at room temperature in darkness. The transmission curves from bottom to top were taken after 0, 1, and 2 days, respectively. The upper curve remained constant on the fourth day.

trans compound, the peak appears at 286–288 mμ. Illumination, preferably with an ultraviolet lamp, is needed for this process.

All diphenyloctatetraenes possessing *cis* double bonds thus far observed are labile (115), and it is therefore understandable that they have never appeared in the course of syntheses. Even with a knowledge of their properties, it has been impossible up to the present to prepare crystalline samples for which the absence of some all-*trans* form could be guaranteed. Chromatographic analysis showed that fresh eluates of the partially-*cis* zones mentioned contained only traces of the all-*trans* isomer which, however, rapidly increased in quantity on standing, even at 5°C., in darkness.

The spontaneous re-isomerization of *2-cis*-diphenyloctatetraene is demon-

strated in figure 41, which shows as a function of time the gradual increase in the light extinction at room temperature.

Such a re-isomerization is especially rapid in ultraviolet light. If a Tswett column containing adsorbed *2-cis* form is irradiated for a few minutes with an ultraviolet lamp, further development splits it into two fluorescing zones. The inside of the corresponding section of the column, however, does not contain all-*trans*-tetraene, which was formed only locally by irradiation of the surface.

I wish to thank Dr. W. A. Schroeder for his very valuable help in preparing the manuscript of the present review. The help of my assistant, Dr. A. Polgár, is also highly appreciated. To Professor Linus Pauling the author is indebted for stimulating discussions. Professor Arthur B. Lamb kindly permitted the reproduction of some drawings which have appeared in the *Journal of the American Chemical Society*.

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[1] Owing to present conditions, this bibliography is not claimed to be exhaustive.

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