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Some Stereochemical Aspects of Polyenes

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Introduction

One hundred and thirty-five years ago a Marseille pharmacist, named POUTET[2], conducted a remarkable experiment. He shook olive oil with a reagent, obtained by dissolving mercury in excess nitric acid, and observed a few hours later that the oil had solidified. Some other vegetable oils remained liquid upon a similar treatment. POUTET thus found a suitable method for the detection of falsifications in commercial olive oil. Of course, he could not have been aware of the fact that he had performed a pioneer experiment in stereochemistry, half a century before VAN T'HOFF and LE-BEL disclosed their fundamental ideas.

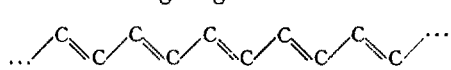
Since olive oil is rich in glycerides including unsaturated fatty acids and since the molecules of most such acids contain at least one double bond in *cis* configuration, POUTET's experiment must be rated as a *cis* → *trans* rearrangement, termed "elaidinization" in modern fat chemistry.

cis-Configuration. *trans*-Configuration[3].

As is well known, VAN T'HOFF's concept of *cis-trans* isomerism was too revolutionary to be accepted without contradiction. Even after a lapse of ten years ANSCHÜTZ[4], a leading organic chemist, rejected the spatial character of the maleic acid–fumaric acid relationship; he assumed that such a "slight" variation could not possibly explain the very different behavior of the two acids.

A similar scepticism was voiced not such a long time ago with reference to the existence of stereoisomeric forms of polyenes.

Although the term "poly-ene" would *a priori* include any compound whose molecules contain more than one double bond in an open chain, the present use of this term is rather generally restricted to "conjugated" systems of alternating single and double bonds:



Whereas many properties of compounds of another type, containing "isolated" double bonds in an open chain, can be understood as the sum of the contributions of the individual double bonds, in contrast, essentially new features appear when the system becomes conjugated. Some authors have suggested[1] that the electrons are "smeared" along such systems, depriving each C=C group of most of its double bond character. This would exclude the existence of *cis* forms. Although this assumption seemed to be confirmed by the almost exclusive occurrence of the all-*trans* configuration both in natural and synthetic polyenes, it was pointed out by KUHN[2] that in the case of the Orleans pigment, bixin, Nature herself has provided us with an example of a *cis* polyene, the character of which has been clarified by KARRER[3].

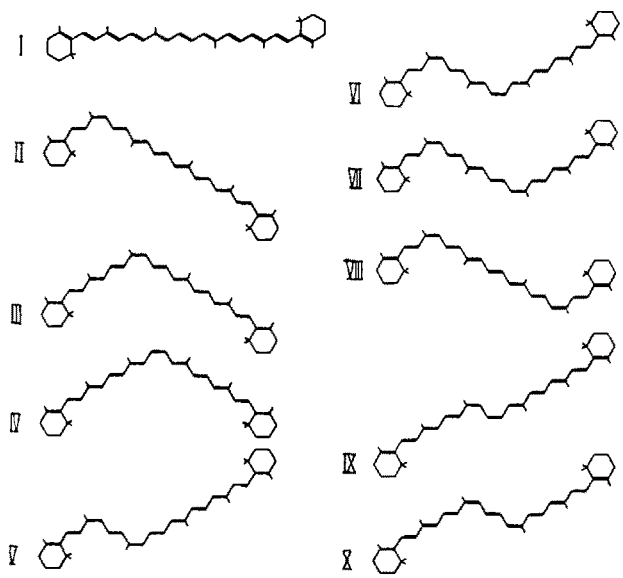


Fig. 1.–A few carbon skeleton models of stereoisomeric β-carotenes: I, all-*trans*; II–IV, mono-*cis*; and V–X, di-*cis* forms.

Today we know that polyenes are generally subject to *cis-trans* isomerization; and a few carbon skeleton models of stereoisomeric β-carotenes (Fig. 1) will suffice to illustrate the spatial versatility of such molecules.

[1] California Institute of Technology, Pasadena.

[2] J. J. E. POUTET, Ann. chim. phys. [2] *12*, 58 (1819).

[3] The prefixes, *cis* and *trans*, were introduced by A. BAEYER, Liebigs Ann. Chem. *245*, 103 (1888).

[4] R. ANSCHÜTZ, Liebigs Ann. Chem. *239*, 161 (1887).

[1] G. WITTIG and W. WIEMER, Liebigs Ann. Chem. *483*, 144 (1930).

[2] R. KUHN, in Freudenbergs Stereochemie (Fr. Deuticke, Leipzig-Wien, 1933), p. 913.

[3] P. KARRER, A. HELFENSTEIN, R. WIDMER, and TH. B. VAN ITALLIE, Helv. chim. Acta *12*, 741 (1929).

Methods of cis-trans Isomerization

The experimental procedures leading from the ordinary or all-*trans* form of a polyene to its *cis* isomers are in principle identical with those having effect on mono- or di-unsaturated compounds. However, instead of a simple product, a complicated mixture of stereoisomers is obtained in which unchanged all-*trans* molecules may or may not predominate. The most frequently applied thermic and photochemical treatments are: keeping a solution at room temperature ("spontaneous stereoisomerization"), refluxing solutions, melting crystals, illumination of solutions with light of suitable wave length or exposure to sunshine. Among the stereo-catalytic methods available at room temperature the very effective treatment with iodine should be emphasized.

It was observed by ANSCHÜTZ[1] in 1878 that the silver salts of both maleic and fumaric acids when reacted with ethyl iodide yielded the same ester, viz. ethyl fumarate. Subsequently, ANSCHÜTZ found that a slight iodine content of the ethyl iodide used in his first experiments had been responsible for this surprising identity, in the sense that the iodine had catalytically converted the maleic into fumaric acid. Silver maleate, upon interaction with iodine-free ethyl iodide, gave exclusively the maleic ester.

Iodine catalysis was introduced into the stereochemistry of C_{40}-polyenes in 1938–1939[2] and it was found that it requires illumination.

In principle, any catalytic treatment of a polyene may induce, besides reversible stereoisomerization, also irreversible processes. The extent of the latter is practically negligible when the iodine treatment is carried out under proper conditions but it may involve a substantial fraction of the compound during acid catalysis, for example. It is still more difficult to steer the process in the direction of *trans* → *cis* rearrangements when a polyene is first converted with boron trihalogenide etherate to a (dark) complex[3] which is eventually broken up by the addition of water or methanol. We have carried out this last step, for example, one minute after the formation of the β-carotene (III) complex and obtained mainly stereoisomers of the carotene; in contrast, the conjugated system underwent shortening and destruction in the course of a longer standing of the complex[4]. Dehydro-β-carotene (IV) is so sensitive that even a one-minute standing of its BF_3-complex results in the formation of conversion products while no stereoisomers appear[5].

Theoretically, it is to be expected that any stereoisomerization would result in the formation of a (quasi-) equilibrium mixture containing all possible "members of the stereoisomeric set". Experiment has shown, however, that in most instances only a few, viz. 3 to 10, molecular forms are preferred and that on the usual laboratory scale most of the possible stereoisomers escape detection.

A successful study of *cis-trans* isomeric polyene mixtures requires thus the solution of the following two problems: (1) the stereoisomeric mixtures must be resolved, and (2) each pure, individual compound must be proven to represent a stereoisomer of the starting material.

Since polyenes are not volatile, the only means for their effective separation is chromatographic analysis. It has been found in this class of compounds that the adsorption affinities are as highly dependent on the spatial configuration as on the chemical structure. Hence, differences in the molecular shape do permit clear separations on the chromatographic column. In order to prove that a zone truly contains a stereoisomer of a given polyene and nothing else, it is eluted, submitted to iodine catalysis and the solution chromatographed again. The all-*trans* zone thus obtained is then mixed with an authentic sample of the same substance and developed on a column ("mixed chromatogram test"). Non-separation indicates identity which should be confirmed by a comparison of the spectra.

Using the methods just outlined, a number of *cis* carotenoids have been prepared, several of them in the crystalline state, and their physical, chemical and biological characteristics (if any) have been determined. Although this development encountered considerable scepticism at first[1], most of our methods and results now appear to have been generally accepted.

Types of Polyenes Investigated

Although a variety of polyene systems still remains to be explored by the stereochemist, marked progress has been made in the following four classes of compounds[2]:

(a) *Non-isoprenic conjugated systems with aromatic end groups:* α, ω-Diphenylpolyenes[3]:

$$C_6H_5 \cdot (CH = CH)_n \cdot C_6H_5$$

($n = 1$: stilbene; $n = 2$: diphenylbutadiene; $n = 3$: diphenylhexatriene; and $n = 4$: diphenyloctatetraene).

[4] R. ANSCHÜTZ, Ber. dtsch. chem. Ges. *11*, 1644 (1889); *12*, 2282 (1879).

[2] L. ZECHMEISTER and P. TUZSON, Nature *141*, 249 (1938); Biochem. J. *32*, 1305 (1938); Ber. dtsch. chem. Ges. *72*, 1340 (1939).

[3] G. N. LEWIS and G. T. SEABORG, J. Amer. Chem. Soc. *61*, 1886 (1939). – H. H. STRAIN, J. Amer. Chem. Soc. *63*, 3448 (1941).

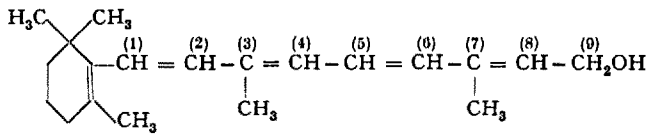
[4] L. WALLCAVE, J. LEEMANN, and L. ZECHMEISTER, Proc. Nat. Acad. Sci. (U.S.A.), *39*, 604 (1953).

[5] L. WALLCAVE and L. ZECHMEISTER, J. Amer. Chem. Soc. *75*, 4495 (1953).

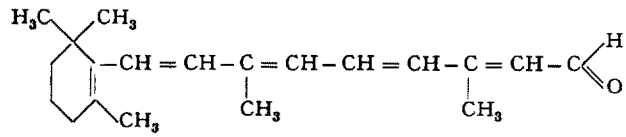
[1] Cf. P. KARRER and J. RUTSCHMANN, Helv. chim. Acta *27*, 1684 (1944).

[2] General survey: L. ZECHMEISTER, Chem. Reviews *34*, 267 (1944).

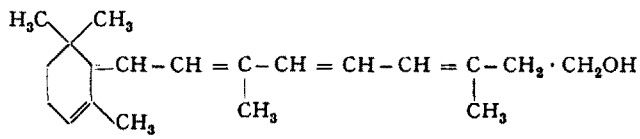
[3] L. ZECHMEISTER and W. H. MCNEELY, J. Amer. Chem. Soc. *64*, 1919 (1942). – L. ZECHMEISTER and A. L. LEROSEN, J. Amer. Chem. Soc. *64*, 2755 (1952). – A. SANDOVAL and L. ZECHMEISTER, J. Amer. Chem. Soc. *69*, 553 (1947). – J. H. PINCKARD, B. WILLE, and L. ZECHMEISTER, J. Amer. Chem. Soc. *70*, 1938 (1948). – Unpublished studies, in collaboration with K. LUNDE and with J. H. PINCKARD.



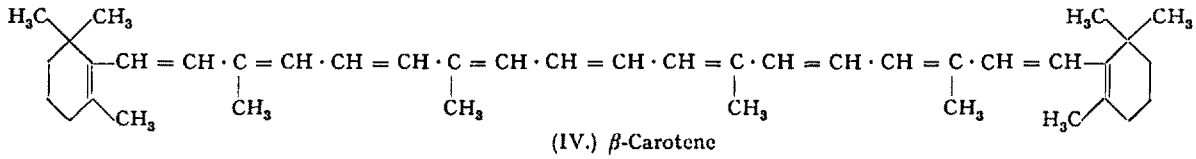
(I.) Vitamin A



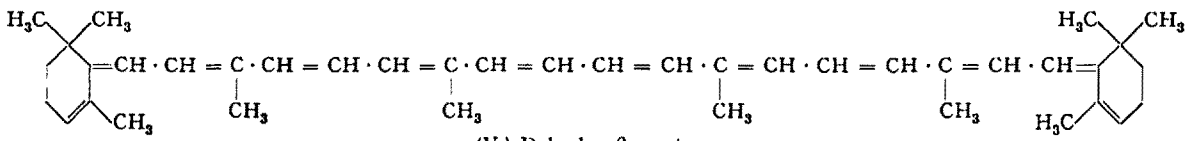
(II.) Retinene



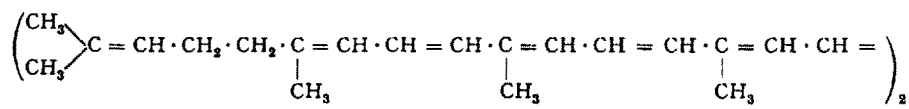
(III.) Retrovitamin A



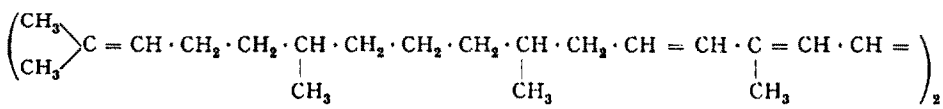
(IV.) β-Carotene



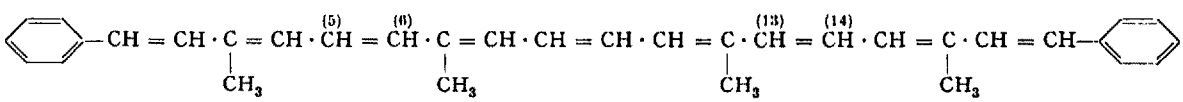
(V.) Dehydro-β-carotene



(VI.) Lycopene



(VII.) Phytofluene (probable formula)



(VIII). Diphenyl-3, 7, 12, 16-tetramethyl-octadecanonaene

Symmetrical azines[1]:

$$C_6H_5 \cdot (CH=CH)_n \cdot CH=N-N=CH \cdot (CH=CH)_n \cdot C_6H_5$$

($n = 1$: cinnamalazine; and $n = 2$: phenylpentadienalazine).

(b) *Non-isoprenic conjugated systems with heterocyclic end groups:* a representative of the cyanine dyes[2].

(c) *Isoprenic conjugated systems with aliphatic or hydroaromatic end groups:* C_{20}–C_{25}-Polyenes: vitamin A and related compounds[3], retinene[4], retro-vitamin A[5] (Formulas I–III), etc.; methylbixin[1]. C_{40}-Polyenes: carotenoid pigments[2] and some colorless, fluorescent polyenes such as phytofluene[3] (Formulas IV–VII).

(d) *Isoprenic conjugated systems with aromatic end groups:* KARRER's synthetic compounds[4] (Formula VIII).

Steric Hindrance and Number of cis-trans Isomers

The formulas (I–VIII) represent all-*trans* compounds. Provided that each of their *trans* double bonds is able to assume *cis* configuration, numerous steric forms can be expected, for example, 16 for vitamin A, 272 for β-carotene, and 1056 for lycopene.

[1] J. DALE and L. ZECHMEISTER, J. Amer. Chem. Soc. *75*, 2379 (1953).

[2] L. ZECHMEISTER and J. H. PINCKARD, Experientia *9*, 16 (1953).

[3] CH. D. ROBESON and J. G. BAXTER, J. Amer. Chem. Soc. *69*, 136 (1947). – J. D. CAWLEY, CH. D. ROBESON, L. WEISLER, E. M. SCHANTZ, N. D. EMBREE, and J. G. BAXTER, Science *107*, 346 (1948). – W. GRAHAM, D. A. VAN DORP, and J. F. ARENS, Rec. Trav. chim. Pays-Bas *68*, 609 (1949). – W. OROSHNIK, G. KARMAS, and A. D. MEBANE, J. Amer. Chem. Soc. *74*, 295, 3807 (1952); *75*, 1050 (1953).

[4] R. HUBBARD, R. I. GREGERMAN, and G. WALD, J. gen. Physiol. *36*, 415 (1953).

[5] W. OROSHNIK, G. KARMAS, and A. D. MEBANE, J. Amer. Chem. Soc. *74*, 295 (1952).

[1] L. ZECHMEISTER and R. B. ESCUE, J. Amer. Chem. Soc. *66*, 322 (1944).

[2] General survey: L. ZECHMEISTER, Chem. Reviews *34*, 267 (1944).

[3] F. J. PETRACEK and L. ZECHMEISTER, J. Amer. Chem. Soc. *74*, 184 (1952).

[4] C. H. EUGSTER, C. F. GARBERS, and P. KARRER, Helv. chim. Acta *35*, 1179 (1952). – C. F. GARBERS, C. H. EUGSTER, and P. KARRER, Helv. chim. Acta *36*, 562 (1953).

Three-dimensional models show, however, that *trans* → *cis* rearrangements cannot take place with equal facility around each double bond, considering the presence of some geometrical hurdles. Three types of such "steric hindrance" have been under discussion so far.

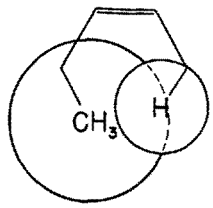


Fig. 2.–Steric hindrance: Overlapping of a hydrogen atom and a methyl group in *cis* isoprenic polyenes. [From: Fortschr. Chem. organ. Naturstoffe *3*, 203 (1939).]

Firstly, it was pointed out by PAULING[1] with reference to isoprenic polyenes that when a carbon atom adjacent to a double bond carries a methyl group, a considerable overlapping of this group and a hydrogen atom is to be expected as a result of *trans* → *cis* isomerization (Fig. 2). Secondly, according to LEWIS *et al.*[2] two H-atoms attached to the two rings in *cis*-stilbene will overlap (Fig. 3); and thirdly, we have shown for terminal-*cis* diphenylpolyenes that a hydrogen atom of the ring, in opposition to the side chain, is spatially hindered by the latter; thus an approximately planar configuration becomes impossible[3] (Fig. 4).

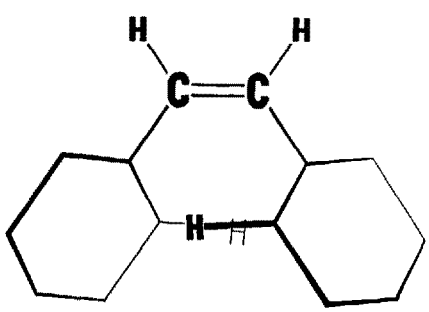


Fig. 3.–Steric hindrance: Overlapping of two ring-hydrogen atoms in *cis*-stilbene. [From: J. Amer. Chem. Soc. *62*, 2973 (1940).]

During the last few years several authors have discussed the existence or non-existence of sterically hindered *cis* polyenes. Recently, several experimental studies have shown that none of the three hindrance types mentioned constitutes an absolute block. Certain hindered *cis* forms do exist and they are not necessarily thermolabile in spite of their relatively high energy content. It should be noted, however, that "hindered" configurations rarely appear upon iodine catalysis; on the contrary, they seem to be very iodine sensitive and disappear from the solution when catalyzed.

[1] L. PAULING, Fortschr. Chem. organ. Naturstoffe *3*, 203 (1939).
[2] G. N. LEWIS, T. T. MAGEL, and D. LIPKIN, J. Amer. Chem. Soc. *62*, 2973 (1940).
[3] L. ZECHMEISTER and A. L. LEROSEN, J. Amer. Chem. Soc. *64*, 2755 (1942).

The following two practical routes have led to some sterically hindered forms: (1) direct introduction of energy by illuminating an all-*trans* polyene; and (2)

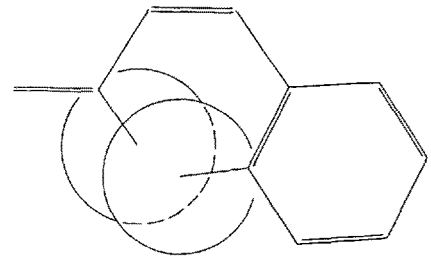


Fig. 4.–Steric hindrance: Overlapping of two hydrogen atoms in a terminal *cis*-diphenylpolyene. [From: J. Amer. Chem. Soc. *64*, 2755 (1942).]

total synthesis *via* the partial reduction of a triple bond (or of a cumulene structure) located at the site of the desired hindered double bond.

In the non-hindered system of maleic-fumaric acids it was demonstrated by WARBURG in 1919 that the *trans* acid is converted into the *cis* form by ultraviolet illumination. An early example in the field of hindered ethylene derivatives is the photochemical conversion, *trans*-stilbene → *cis*-stilbene, as reported by STOERMER[1] and by LEWIS[2].

In the stilbene and diphenylbutadiene molecules all aliphatic double bonds are of "hindered" nature, and in diphenylhexatriene all but that one in the center. When a dilute solution of all-*trans*-diphenylbutadiene was kept in diffuse daylight, rearrangement to a hindered *cis* form took place; however, the all-*trans* configuration was recovered almost quantitatively upon iodine catalysis (Fig. 5)[3]. Recently, a *cis* cinnamalazine for which a hindered configuration is very probable was obtained, in collaboration with J. DALE, by prolonged exposure of *trans* solutions to intense sunshine or by melting crystals[4]. The *cis*-diphenylbutadienes had been prepared by the partial reduction of the corresponding acetylene derivatives half a century ago (STRAUS)[5]. In our laboratory J. H. PINCKARD and K. LUNDE have obtained some hindered *cis* forms of diphenyl-octatetraene and -hexatriene by direct isomerization of the respective all-*trans* compounds (unpublished).

In the field of isoprenic polyene systems the first case of a hindered *cis* isomer was reported by OROSHNIK[6]. In the class of carotenoid pigments the well-

[1] R. STOERMER, Ber. dtsch. chem. Ges. *42*, 4865 (1909). – R. STOERMER and L. PRIGGE, Liebigs Ann. Chem. *409*, 20 (1915).
[2] G. N. LEWIS, T. T. MAGEL, and D. LIPKIN, J. Amer. Chem. Soc. *62*, 2973 (1940). – G. N. LEWIS and J. BIGELEISEN, J. Amer. Chem. Soc. *65*, 2102, 2107 (1943).
[3] A. SANDOVAL and L. ZECHMEISTER, J. Amer. Chem. Soc. *69*, 553 (1947). – J. H. PINCKARD, B. WILLE, and L. ZECHMEISTER, J. Amer. Chem. Soc. *70*, 1938 (1948).
[4] J. DALE and L. ZECHMEISTER, J. Amer. Chem. Soc. *75*, 2379 (1953).
[5] F. STRAUS, Liebigs Ann. Chem. *342*, 190 (1905).
[6] W. OROSHNIK, G. KARMAS, and A. D. MEBANE, J. Amer. Chem. Soc. *74*, 295, 3807 (1952); *75*, 1050 (1953).

known total-synthetic work[1] carried out by the schools of KARRER in Zürich and INHOFFEN in Braunschweig made it possible to prepare, *via* acetylene derivatives, all types of *cis* isomers, including some

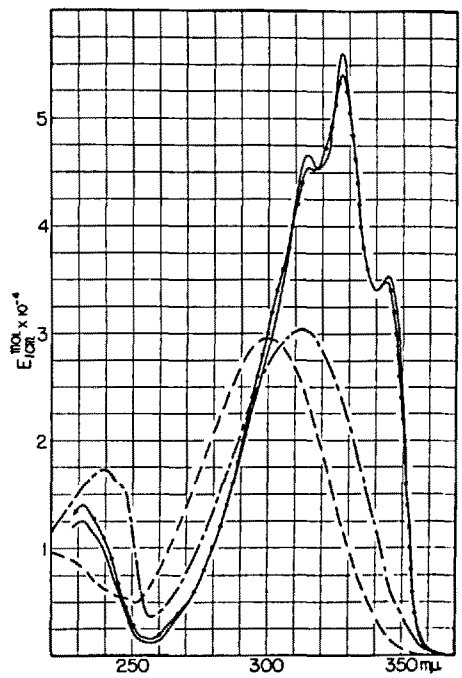


Fig. 5.–Molecular extinction curves of stereoisomeric diphenylbutadienes, in hexane: ———, *trans-trans*; —·—, *cis-trans*; and – – – – –, *cis-cis*; –·–·–·–, after iodine catalysis of any of the three stereoisomers. [From: J. Amer. Chem. Soc. *70*, 1938 (1948).]

hindered forms, with determined absolute configurations. The importance of similar synthetic compounds, especially of KARRER's hindered isoprenic polyenes containing aromatic end groups, in the evaluation of some spectral phenomena will be indicated below[2].

Steric Configuration and the Main Spectral Band

While chromatography constitutes a unique tool for the separation and isolation of *cis-trans*-isomeric polyenes, spectroscopy is the choice method of rapid characterization of a pure, individual spatial form. Although the exact relationship between the molecular shape and spectrum is not yet fully understood, some interesting correlations have been secured.

The extinction curve of a polyene contains, first of all, a main band, located within or near the visible region and showing more or less fine structure. According to classical electromagnetic theory it represents the vibration of mobile "unsaturation" electrons along the whole length of the conjugated system. Resonance stabilization at the single bonds keeps an all-*trans* chromophore approximately coplanar[1]. Within a given stereoisomeric set the all-*trans* compound occupies a privileged position, in the sense that its main spectral band shows a longer wave length maximum and a higher extinction value than the band of any *cis* isomer. Thus, the straight molecular form of an all-*trans* pigment brings about the strongest possible color within the set. Since the all-*trans* configuration is by far preponderant in the vegetable kingdom, Nature when producing color makes the best possible use of a given conjugated system. The older concept that the color of a polyene is determined only by the length of the chromophore had to be abandoned, since it did not envisage the profound influence of the steric configuration on the spectrum.

Let us now imagine the following experiment: Starting from an all-*trans* polyene we perform a stepwise *trans* → *cis* rearrangement, proceeding from one double bond to the next. We assume that each such step will cause some loss of fine structure in the main band and a certain displacement of the main maximum towards shorter wave lengths. No theory is available at the present time on the basis of which one could predict the extent of these shifts. An experimental approach was possible, however, by taking the extinction curves of numerous chromatographically purified *cis* carotenoids. It was observed that in many instances the location on the wave length scale of the respective main maxima differed from that of the all-*trans* compound by 5 mμ or 10 mμ or 15 mμ (± 1 mμ; in hexane solution). Although these data were interpreted by assuming, respectively, mono*cis*, di*cis*, and tri*cis* configurations, it was stressed nine years ago that "nothing definite is known about the additivity of spectral differences and their dependence on the relative position of the double bond concerned"[2]. Hence, considerable aberrations from the observed "regularity" had to be expected in special instances.

Since the quoted statement was made, some pertinent new spectral data have appeared in the literature referring to such *cis* compounds whose configurations had been determined by total synthesis. INHOFFEN and his colleagues[3] reported that their central mono-*cis*-β-carotene (carbon skeleton: Fig. 1; there Model IV) differed from all-*trans*-β-carotene by 3 mμ only in the location of the main maximum. This behavior might be explained by the unique structural position

[1] P. KARRER and C. H. EUGSTER, C. r. Acad. Sci. *250*, 1920 (1950), and numerous papers in the Helv. chim. Acta. – H. H. INHOFFEN, F. BOHLMANN, K. BARTRAM, and H. POMMER, Chem. Z. *74*, 285 (1950), and numerous papers in Liebigs Ann. Chem. Cf. also N. A. MILAS, P. DAVIS, I. BELIČ, and D. A. FLEŠ, J. Amer. Chem. Soc. *72*, 4844 (1950).

[2] *Addendum:* C. F. GARBERS and P. KARRER, Helv. chim. Acta *36*, 828 (1953), have synthesized, quite recently, several hindered *cis* lycopenes. These could not be obtained in crystalline form and have been characterized on the basis of spectral readings. Their spectra are very similar to some observed in the series of poly*cis*-lycopenes.

[1] L. PAULING, Fortschr. Chem. organ. Naturstoffe *3*, 203 (1939). – L. ZECHMEISTER, A. L. LEROSEN, W. A. SCHROEDER, A. POLGÁR, and L. PAULING, J. Amer. Chem. Soc. *65*, 1940 (1943).

[2] L. ZECHMEISTER, Chem. Reviews *34*, 267 (1944).

[3] H. H. INHOFFEN, F. BOHLMANN, K. BARTRAM, G. RUMMERT, H. POMMER, F. WESTPHAL, and G. LINHOFF, Liebigs Ann. Chem. *570*, 54 (1950).

of the central double bond, the only one which is not connected with C-methyl groups.

On the other hand, it was observed by KARRER *et al.*[1] when studying the system represented in formula (VIII) that the rearrangement, all-*trans* → 5,6-(hindered) mono*cis*, caused an extraordinarily large spectral shift. At the same time the curve flattened out markedly, although the fine structure did not disappear. However, the presence of two hindered *cis* double bonds (5,6- and 13,14-) did practically demolish the fine structure.

In the much shorter diphenylbutadiene system[2] such total loss of fine structure appeared upon a single rearrangement from all-*trans* to hindered *cis* (Fig. 4).

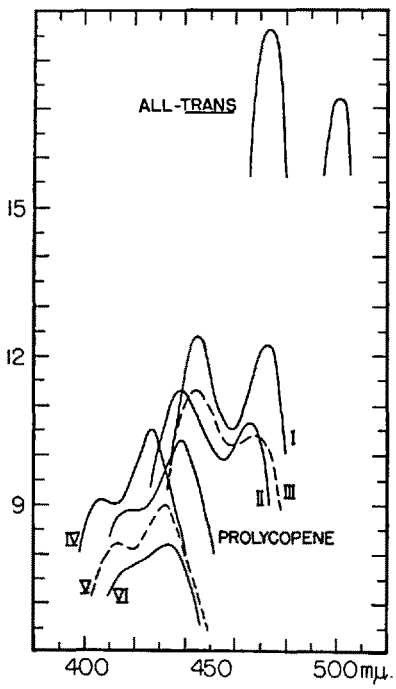


Fig. 6.–Molecular extinction curves, in hexane, of all-*trans*-lycopene and seven poly*cis* lycopenes at their main maxima. [From: J. Amer. Chem. Soc. *69*, 1930 (1947).]

These relationships are also illustrated by some observations made on naturally occurring *cis* carotenoids[3]. Evidently, under the influence of special genetic factors[4], some plant varieties deviate from the "all-*trans* rule" and steer the pigment biosynthesis towards *cis* configurations. Thus, we were able to isolate from the berries of *Pyracantha angustifolia* several "poly-*cis*" lycopenes in crystalline form[1]. When inspecting their spectral curves as given in Figure 6, one would not assume *a priori* that they all refer to the same chemical constitution. The sharp dependence of the extent of fine structure on the molecular form is remarkable indeed. The main band of central mono *cis*-lycopene (Fig. 7) lies between those of the all-*trans* and the poly*cis* forms.

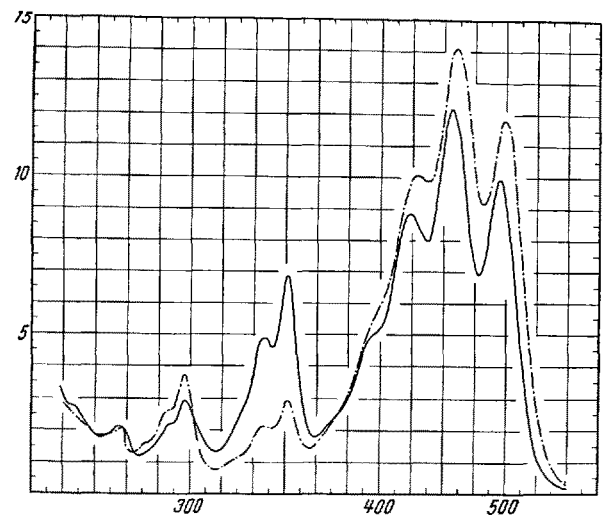


Fig. 7.–Molecular extinction curve, in hexane, of neolycopene A (very probably central mono*cis*-lycopene): ———, before iodine catalysis; and –·–·–, after iodine catalysis. [From: J. Amer. Chem. Soc. *65*, 1940 (1943).]

The poly*cis* compound "prolycopene" when catalyzed with relatively large amounts of iodine (1% of the pigment) yielded the expected stereochemical equilibrium mixture almost instantaneously, in which all-*trans*-lycopene (V) was preponderant (Fig. 8). However,

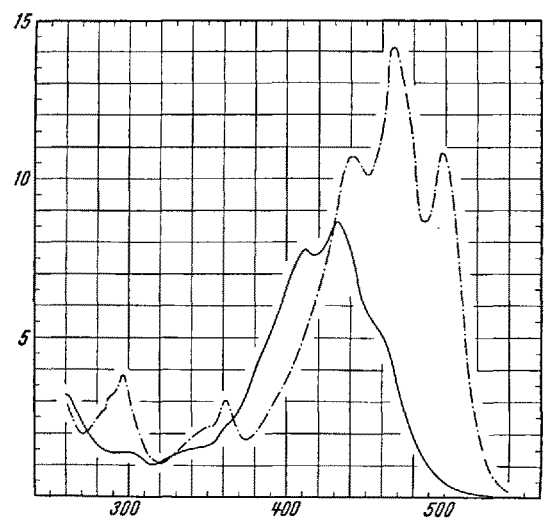


Fig. 8.–Molecular extinction curve, in hexane, of a poly*cis* lycopene: ———, before iodine catalysis; and ·–·–·–, after iodine catalysis. [From: J. Amer. Chem. Soc. *65*, 1940 (1943).]

[1] C. H. EUGSTER, C. F. GARBERS, and P. KARRER, Helv. chim. Acta *35*, 1179 (1952). – C. F. GARBERS, C. H. EUGSTER, and P. KARRER, Helv. chim. Acta *35*, 1850 (1952); *36*, 562 (1953).

[2] A. SANDOVAL and L. ZECHMEISTER, J. Amer. Chem. Soc. *69*, 553 (1947). – J. H. PINCKARD, B. WILLE, and L. ZECHMEISTER, J. Amer. Chem. Soc. *70*, 1938 (1948).

[3] A. L. LEROSEN and L. ZECHMEISTER, J. Amer. Chem. Soc. *64*, 1075 (1942). – L. ZECHMEISTER and W. A. SCHROEDER, J. Amer. Chem. Soc. *64*, 1173 (1942).

[4] L. ZECHMEISTER and F. W. WENT, Nature (London) *162*, 847 (1948). – J. W. PORTER and F. P. ZSCHEILE, Arch. Biochem. *10*, 537, 547 (1946). – J. W. PORTER and R. E. LINCOLN, Arch. Biochem. *27*, 390 (1950). – H. H. TROMBLY and J. W. PORTER, Arch. Biochem. Biophys. *43*, 443 (1953).

[1] L. ZECHMEISTER and J. H. PINCKARD, J. Amer. Chem. Soc. *69*, 1930 (1947).

when the quantity of the catalyst was decreased 1000 times, for example, the process was slowed down and a chromatogram, taken after a few minutes' iodine treatment, contained a dozen stereoisomers whose spectral maxima were located between those of the catalyzed poly*cis* compound and the all-*trans* form. Two of the products, as judged on the basis of their spectra, seemed to contain even more *cis* double bonds than the starting material.

Steric Configuration and cis-Peak Effect

It was observed ten years ago that when an ordinary (all-*trans*) carotenoid undergoes stereoisomerization, involving as described the decrease and shift of its main spectral band, at the same time a new maximum, the so-called "*cis*-peak" grows out in a shorter wave length region (Fig. 9)[1]. As a rule the *cis* forms of a given polyene having a *cis*-peak shows it at the same wave length; however, the height of the peak is subject to great variations and is a characteristic constant of each stereoisomeric form (Fig. 10). The distance as measured in mμ on the wave length scale between the *cis*-peak and the maximum of the main band is practically constant for a given conjugated system; it is shorter in shorter systems.

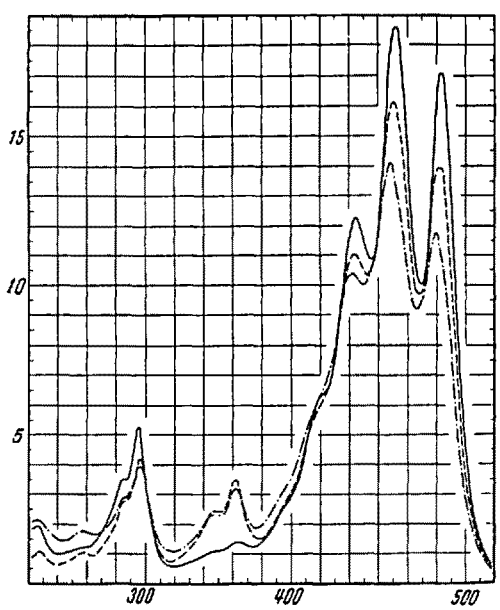


Fig. 9.–Molecular extinction curve of lycopene, in hexane: ———, all-*trans* form; – – – – –, after refluxing for 45 min; and ·–·–·–, after iodine catalysis. [From: J. Amer. Chem. Soc. *65*, 1940 (1943).]

According to PAULING *et al.*[2] the *cis*-peak arises from the oscillation of the electrons from the two ends of a conjugated system toward the center and from the center toward the two ends. The nature of this oscillation is such that it gives rise to no dipole moment and hence to no corresponding spectral band for the all-*trans* molecule. However, bent molecules do have a dipole moment that is perpendicular to the straight line between the ends of the conjugated system, instead of parallel to it. The central mono*cis* isomer would show the highest *cis*-peak of all. As a rough approximation the intensity of the *cis*-peak of a mono*cis* compound, for example, can be taken proportional to the square of the distance between the center of the conjugated system and the mid-point of the straight line between its two ends. Hence, spectral observations in the *cis*-peak region may well contribute to the steric diagnosis of a compound.

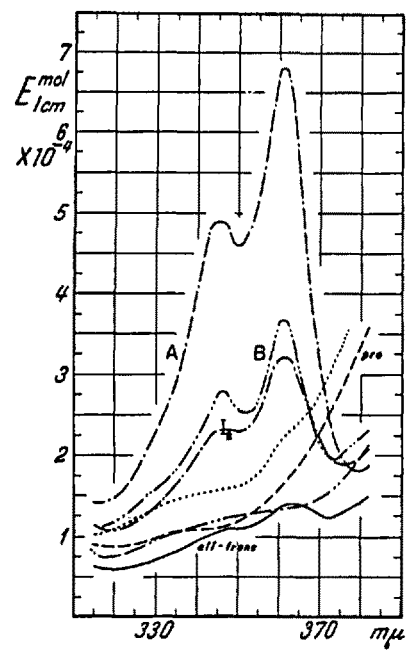


Fig. 10.–Molecular extinction curves in the *cis*-peak region, in hexane of several stereoisomeric lycopenes. (I_2 = quasi equilibrium mixture obtained upon iodine catalysis.) [From: J. Amer. Chem. Soc. *66*, 137 (1944).]

Since polyenes containing many *cis* double bonds have an overall straight molecular shape, comparable to that of the corresponding all-*trans* compound (Fig. 11), their spectral curves should not show any marked *cis*-peak. This postulate has been first verified by using naturally occurring "prolycopene" (Fig. 10). The influence of hindered *cis* configurations on the presence or absence of increased light absorption in the *cis*-peak region cannot yet be formulated in a general manner.

Recently, the *cis*-peak effect has also been observed when polyenes different from carotenoid pigments were used, such as phytofluene[1], diphenyloctatetraene[2]

[1] L. ZECHMEISTER and A. POLGÁR, J. Amer. Chem. Soc. *65*, 1522 (1943).

[2] L. ZECHMEISTER, A. L. LEROSEN, W. A. SCHROEDER, A. POLGÁR, and L. PAULING, J. Amer. Chem. Soc. *65*, 1940 (1943).

[1] F. J. PETRACEK and L. ZECHMEISTER, J. Amer. Chem. Soc. *74*, 184 (1952). – B. K. KOE and L. ZECHMEISTER, Arch. Biochem. Biophys. *46*, 100 (1953).

[2] In collaboration with J. H. PINCKARD (unpublished).

or cinnamalazine[1] (Figs. 12–14). There is, however, one exception in the carotenoid series[2]: No clear *cis*-peak phenomenon appeared in the set of dehydro-*β*-carotene (IV) and a few similar compounds with "retro" structure[3], i.e. containing a single bond in the center but double bonds between the hydroaromatic rings and the main aliphatic chain. A theoretical interpretation of this "anormal" behavior is not available at the present time.

Steric Configuration and Infrared Spectra

Considering the nature of the vibrations responsible for the spectral bands observed in the infrared region, it was expected that the shape of the infrared curve would also depend on the spatial configuration of a polyene. Recently, Mag. KAARE LUNDE and the present writer have carried out pertinent studies, as yet unpublished, whose results can be summarized as follows.

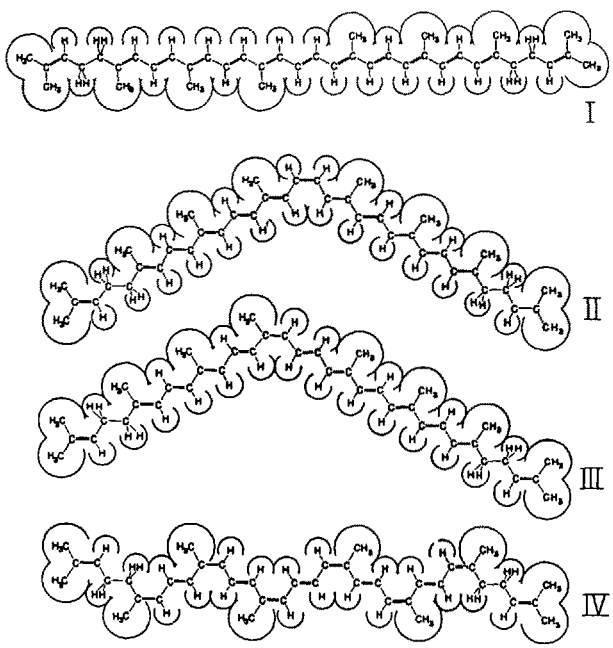


Fig. 11.–Molecular models of some stereoisomeric lycopenes: I, all-*trans*; II, central mono*cis*; III, another mono*cis*; and IV, a poly*cis* form. [From: J. Amer. Chem. Soc. *65*, 1940 (1943).]

Diphenylbutadiene may assume three configurations, viz. *trans-trans*, *cis-trans*, and *cis-cis*. The two *cis* isomers show in carbon tetrachloride solution around 7·25 μ a band that does not appear in the *trans-trans* curve. A similar statement is valid for the region, 12·5 μ – 15 μ (two "*cis*-peaks"; cyclohexane solution). On the other hand, between 10·0 μ and 10·6 μ, a typical "*trans*-peak" is observed that decreases and then disappears during the stepwise rearrangement, *trans-trans* → *cis-trans* → *cis-cis*.

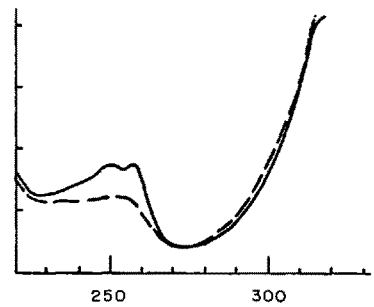


Fig. 12.–*Cis*-peak effect in the phytofluene set: Extinction curves, in hexane: ———, a *cis* form occuring in tomatoes, before iodine catalysis; and – – –, after iodine catalysis. [From: J. Amer. Chem. Soc. *74*, 184 (1952).]

The results obtained when some other representatives of the diphenylpolyene series were used are consistent with those just mentioned.

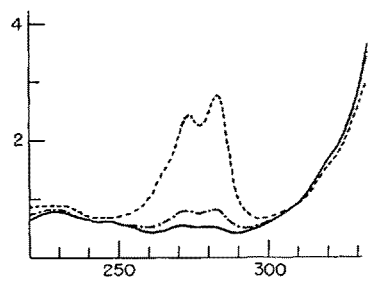


Fig. 13.–*Cis*-peak effect in the diphenyloctatetraene set, in hexane: ———, all-*trans* form; – – – –, a *cis* form; and ·–·–·, curve after iodine catalysis (unpublished).

In the field of the carotenoids several *cis* forms of α-carotene, *β*-carotene, *γ*-carotene, lycopene, and certain hydroxylated compounds have been tested. We are obliged to Professor H. H. INHOFFEN of the Technische Hochschule in Braunschweig for a sample of his

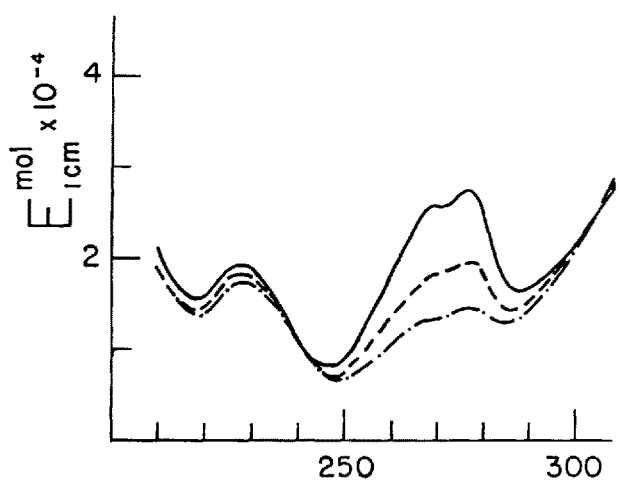


Fig. 14.–*Cis*-peak effect in the cinnamalazine set, in hexane: Extinction curves of *cis*-I cinnamalazine: ———, fresh solution; – – – –, after 150 min illumination with a daylight lamp; and ·–·–·, after exposure to sunshine. [From: J. Amer. Chem. Soc. *75*, 2379 (1953).]

[1] J. DALE and L. ZECHMEISTER, J. Amer. Chem. Soc. *75*, 2379 (1953).

[2] L. ZECHMEISTER and L. WALLCAVE, J. Amer. Chem. Soc. *75*, 5341 (1953).

[3] Cf. W. OROSHNIK, G. KARMAS, and A. D. MEBANE, J. Amer. Chem. Soc. *74*, 295 (1952).

synthetic central mono*cis*-β-carotene[1]. This well-crystallized compound of known configuration shows a strong absorption maximum at 12·84 μ (Fig. 15) that is missing in the corresponding all-*trans* curve and, indeed, could not be observed in any other all-*trans* spectrum so far.

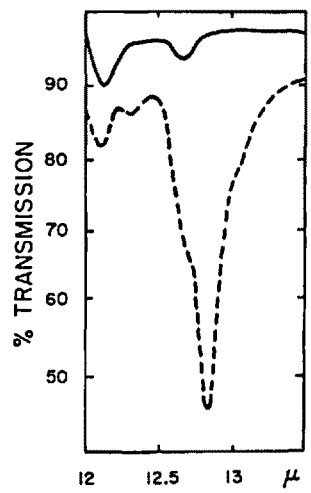


Fig. 15.–Infrared spectral curves in cyclohexane (1 mm NaCl cell): ———, 0·50% solution of all-*trans*-β-carotene; and – – – –, 1·0% solution of synthetic central mono*cis*-β-carotene (unpublished).

The 7·25 μ band included in the two *cis*-diphenylbutadiene curves is also present, with variable intensity, in the spectra of all *cis* carotenoids tested so far and belonging to various stereoisomeric sets. There is, however, one exception: This maximum is missing in the spectrum of central mono*cis*-β-carotene whose molecular symmetry is comparable to that of the corresponding all-*trans* compound.

None of the all-*trans* pigments studied showed maxima either in the 7·25 μ or in the 13 μ region. A 13 μ band did appear in the spectra of some *cis* carotenoids.

It can be predicted with safety that comparative readings taken with the infrared spectrophotometer will become increasingly useful tools in the stereochemical exploration of polyenes. Infrared spectra may extend and complete the information gained in the visible and ultraviolet regions.

Biological Significance of cis-trans Isomerism in C_{20}-Systems

The dependence of the properties of stereoisomeric polyenes on the molecular shape is, as we have seen, so sharp that spatial configurations, especially of vitamins A and of some carotenoids, were expected to influence certain biological processes.

The vitamin A molecule (I) contains four aliphatic double bonds and should occur in 16 spatial forms, four of which are unhindered. "Ordinary" vitamin A has the all-*trans* configuration; however, according to ROBESON and BAXTER[1], one third of many fish liver oils consists of a crystallizable Δ^5-*cis* isomer termed neovitamin A. In some red tuna oils more than half of the vitamin was found in the *cis* configuration[2]. Likewise, synthetic A-concentrates contain substantial amounts of the neovitamin. Evidently, a catalytic interconversion, *trans* vitamin $\rightleftarrows$ *cis*-vitamin(s) takes place both *in vitro* and *in vivo*. Physiologically speaking, what we term vitamin A is a mixture of *cis-trans* isomers[3] and displays a weaker action in the body than would the pure all-*trans* compound. The biopotency of neovitamin A amounts to 70–80 % of the *trans* form[4].

Both structurally and biologically closely related to vitamin A is retinene which, according to MORTON[5], is the aldehyde (II) corresponding to vitamin A. This compound plays a key role in the chemistry of the "visual cycle" which has been the object of successful investigations carried out by WALD and his school and extending over two decades[6]. According to WALD, vertebrate rod vision involves the cleavage and resynthesis of the "visual purple", termed rhodopsin, a polyene-proteid which under the influence of light dissociates into retinene and the protein opsin. In the retina most of the retinene is converted into vitamin A from which rhodopsin is built up again, thus closing the cycle.

Recently, rhodopsin has been synthesized *in vitro* by HUBBARD and WALD[7], who made use of a vitamin A concentrate, opsin, liver alcohol dehydrogenase, and cozymase. When the vitamin concentrate was replaced by pure crystalline all-*trans*-vitamin A, surprisingly enough, no rhodopsin was formed. However, when the authors converted the *trans* vitamin, by means of iodine catalysis, into a mixture of stereoisomers, the mixture did effect the rhodopsin synthesis[8]. Evidently, this vitally important biological process requires a *cis* configuration of the polyene component.

After having prepared *in vitro* five crystalline stereoisomeric *cis* retinenes, the same authors have

[1] H. H. INHOFFEN, F. BOHLMANN, K. BARTRAM, G. RUMMERT, H. POMMER, F. WESTPHAL, and G. LINHOFF, Liebigs Ann. Chem. *570*, 54 (1950).

[1] CH. D. ROBESON and J. G. BAXTER, J. Amer. Chem. Soc. *69*, 136 (1947). For a synthetic 3-*cis*-vitamin A, cf. W. GRAHAM, D. A. VAN DORP and J. F. ARENS, Rec. Trav. chim. Pay-Bas *68*, 609 (1949). Survey: J. G. BAXTER, Fortschr. Chem. organ. Naturstoffe *9*, 41 (1952).

[2] P. MEUNIER and J. JOUANNETEAU, Bull. Soc. chim. biol. *30*, 260 (1948).

[3] J. D. CAWLEY, CH. D. ROBESON, L. WEISLER, E. M. SHANTZ, N. D. EMBREE, and J. G. BAXTER, Science *107*, 346 (1948).

[4] P. L. HARRIS, S. R. AMES, and J. H. BRINKMANN, J. Amer. Chem. Soc. *73*, 1252 (1951).

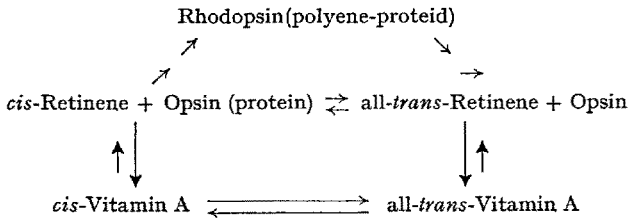
[5] R. A. MORTON, Nature (London) *153*, 69 (1944).

[6] General Survey: G. WALD, *The Chemical Evolution of Vision*, The Harvey Lectures *41*, 117 (1945–1946).

[7] R. HUBBARD and G. WALD, Proc. Nat. Acad. Sci. (U.S.A.) *37*, 69 (1951). – G. WALD, Science *113*, 287 (1951).

[8] R. HUBBARD and G. WALD, Science *18*, 60 (1952); J. gen. Physiol. *36*, 269 (1952–1953).

demonstrated that the particular spatial form liberated from rhodopsin during the cycle has a molecular shape different from that which is required for building up rhodopsin. "The stereoisomerization of retinene or of the corresponding vitamin A is an integral and necessary component of the rhodopsin cycle." Hence, it must constitute a normal function of the body. Very probably, the active isomer is supplied to the eye continuously by the blood stream. The simplified outlines of the visual cycle taking place in the retina are formulated in *Scheme 1*.



Scheme 1. Visual cycle.

Biological Significance of cis-trans-Isomerism in C_{40}-Systems

Our present knowledge that the carotenes represent provitamins A and are (in part) converted in the mammal body into the vitamin by a split in the center of their molecules[1],

$$C_{40}H_{56} \rightarrow 2\,C_{20}H_{29} \cdot OH$$

can be traced back to some basic observations made by STEENBOCK *et al.*[2] and by MOORE[3].

Much later, when the phenomena of polyene stereoisomerization were detected, the question arose how far the biopotencies of the four most common naturally occurring provitamins A, viz. α-carotene, β-carotene, γ-carotene, $C_{40}H_{56}$, and cryptoxanthin, $C_{40}H_{55}$·OH, can be influenced by bending their straight all-*trans* molecules. For the contributions of several authors to this field we may refer to the literature[4].

Systematic investigations into the problem mentioned have been carried out in close collaboration with Dr. H. J. DEUEL, Jr. of the University of Southern California and his group[5]; and recently, the results were discussed in two survey articles[6].

[1] P. KARRER, R. MORF, and K. SCHÖPP, Helv. chim. Acta *14*, 1036, 1431 (1931).

[2] H. STEENBOCK, M. T. SELL, E. M. NELSON, and M. V. BUELL, J. Biol. Chem. *46*, Proc. XXXII (1921).

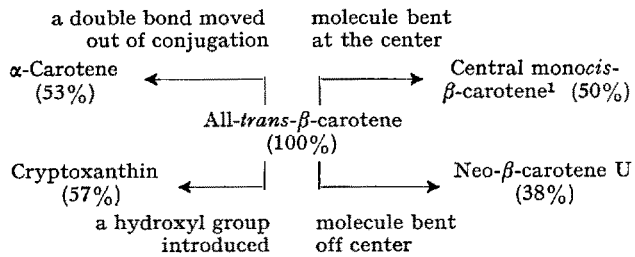
[3] TH. MOORE, Lancet *217*, 380 (1929); Biochem. J. *23*, 803, 1267 (1929).

[4] A. E. GILLAM and M. S. EL RIDI, Biochem. J. *30*, 1735 (1936); the same authors and K. KON, Biochem. J. *31*, 1605 (1937). Later contributions: A. R. KEMMERER and G. S. FRAPS, J. Biol. Chem. *161*, 305 (1945); Ind. Eng. Chem. anal. Ed. *15*, 714 (1943). – B. W. BEADLE and F. P. ZSCHEILE, J. Biol. Chem. *144*, 21 (1942).

[5] Arch. Biochem. Biophys. *5* (1944). – *40* (1952); recent. communication: H. J. DEUEL, Jr., H. H. INHOFFEN, J. GANGULY, L. WALLCAVE, and L. ZECHMEISTER, Arch. Biochem. Biophys. *40*, 352 (1952).

[6] L. ZECHMEISTER, *Stereoisomeric Provitamins A*. Vitamins and Hormones *7*, 57 (1949); *Some Biochemical Studies Based on Chromatographic Methods*. The Harvey Lectures *47*, 221 (1951–1952).

As is well known, the bioeffect of a given provitamin A can be determined quantitatively on the basis of the total gain-in-weight of rats or chicks receiving, after a depletion period, a certain daily dose (micrograms) of the carotenoid tested. Since all-*trans*-β-carotene induces the highest weight increase, its effect is conveniently set equal to 100%. This potency can be substantially decreased either by some structural or by stereochemical variations in the molecules. Significantly, both types of alterations result in a biological weakening of the same order of magnitude as shown



Scheme 2. Weakening of the bioeffect of all-*trans*-β-cartotene by some chemical reactions and spatial rearrangements.

in *Scheme 2* (the figures represent relative provitamin A potencies).

The figures given in Scheme 2 are, evidently, resultants of many partial bio-processes. Among other factors, they seem to have been influenced by the extent to which a certain molecular shape may be fitted into an enzyme system. Of course, it would be temptingly simple to assume that in the body each *cis* provitamin must first be stereoisomerized to the corresponding all-*trans* form, before it can be subjected to the enzymatic attack. However it has been found recently that a naturally occurring *cis* pigment termed pro-γ-carotene shows a bio-potency equal to, or even stronger than, that of all-*trans*-γ-carotene[2]. Consequently, it seems improbable that the numerous *cis* provitamins known should all be inactive *per se*, and that absolute stereochemical bio-specificity would prevail in the enzymatic cleavage system in favor of the all-*trans* configuration.

Conclusion

It was attempted to demonstrate to the non-specialist reader that the class of open-chain polyenes constitutes an exceptionally good example of the strong influence of the molecular shape on the characteristics of a compound. The richness in steric possibilities, unique in the realm of low-molecular substances, offers various points of attack to the investigator but also involves considerable difficulties. These can be,

[1] L. ZECHMEISTER, H. J. DEUEL, Jr., H. H. INHOFFEN, J. LEEMANN, S. M. GREENBERG, and J. GANGULY, Arch. Biochem. Biophys. *36*, 80 (1952).

[2] S. M. GREENBERG, C. E. CALBERT, J. H. PINCKARD, H. J. DEUEL, Jr., and L. ZECHMEISTER, Arch. Biochem. Biophys. *24*, 31 (1949).

in part, overcome by the combination of such different methods as selective adsorption, iodine catalysis, spectroscopy, total synthesis, and bioassays. It is hoped that the field under review may attract the interest of some physicists, chemists and biologists.

Perhaps this writer may close the present incomplete survey of a limited field with a quotation referring to VAN T'HOFF's monumental work that has laid the foundation to all subsequent stereochemical studies: «... je crois qu'il y a là une voie nouvelle dans laquelle il est bon de s'engager, avec prudence sans doute, mais avec une persévérance que les résultats déjà entrevus paraissent justifier[1].»

Zusammenfassung

Polyene, die ein konjugiertes Doppelbindungssystem in offener Kette enthalten, können zahlreiche raumisomere Formen annehmen. In den meisten zu dieser Klasse gehörenden Naturstoffen sowie in Produkten der Synthese wiegt das geradlinige «all-*trans*»-Isomer vor, das aber durch thermische oder photochemische Eingriffe oder durch Jodkatalyse in ein kompliziertes Gemisch von *cis-trans*-Formen umgelagert werden kann. Derartige Gemische lassen sich chromatographisch in ihre Bestandteile zerlegen. Diese Beobachtungen gelten sowohl für isoprenoide Verbindungstypen (carotinoide Farbstoffe, Phytofluen, Vitamin A, Retinen) als auch für Systeme mit unverzweigtem Kohlenstoffgerüst (Diphenylpolyene, Azine).

Die *trans* → *cis*-Umlagerung hat in vielen Fällen eine räumliche Hinderung zu überwinden, deren Grundtypen besprochen werden.

Durch Änderung der stereochemischen Konfiguration eines Polyens werden seine spektroskopischen Merkmale tiefgehend beeinflusst, und zwar im sichtbaren, im ultravioletten und im infraroten Wellenlängengebiet. Der «*cis*-peak-Effekt» dient als ein Mass der Biegung der Moleküle.

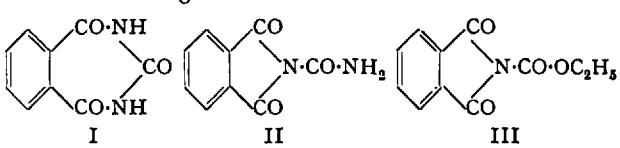
Auch in gewissen biologischen Vorgängen spielt *cis-trans*-Isomerie eine wichtige Rolle; so ist zum Beispiel für den Sehakt ein bestimmter stereo-spezifischer Teilvorgang eine Voraussetzung. Ferner büssen Provitamine A einen grossen Teil ihrer Aktivität im Organismus ein, wenn ihre *trans*-Moleküle morphologische Variationen erleiden. Auch hier zeigt sich ein enger Zusammenhang zwischen Biopotenz und Molekularform.

[1] From a letter sent by WURTZ to VAN T'HOFF in 1877; cf. E. COHEN, *Jacobus Henricus van t'Hoff* (Akad. Verlagsges., Leipzig 1912).

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

Die Konstitution des Phthalylharnstoffs

Phthalylharnstoff, der durch Einwirkung von Phosphoroxychlorid auf Phthalursäure[1] oder von Phthalylchlorid auf Harnstoff[2] gewonnen werden kann, wurde bisher immer als I formuliert, obwohl seine Herstellung, Eigenschaften und Reaktionen auch mit Formel II in bestem Einklang stehen.



I II III

Wir konnten im Laufe unserer Versuche eine Beobachtung machen, die für Formel II sprach. Als sich nämlich das von HELLER und JACOBSOHN[3] beschriebene Phthalylurethan (III) gegenüber Ammoniak als sehr reaktionsfähig erwies, indem es in alkoholischer Lösung mit überschüssigem konzentriertem wässerigem Ammoniak schon bei Zimmertemperatur unter Ringöffnung *Phthalamid* gab, prüften wir auch Phthalylharnstoff auf sein Verhalten gegenüber Ammoniak unter den gleichen Bedingungen. Es ergab sich, dass Phthalylharnstoff, ganz gleich dem Phthalylurethan, mit Ammoniak unter Bildung von Phthalamid reagierte. Auch mit Hydrazinhydrat reagierten beide Verbindungen ganz analog unter Bildung von *Phthalhydrazid*.

Einen Beweis für die Richtigkeit der Formel II lieferte uns schliesslich das Ergebnis der Umsetzung von Phthalylharnstoff mit Xanthydrol. Bekanntlich setzen sich monosubstituierte und asymmetrisch disubstituierte Harnstoffe mit Xanthydrol in Essigsäure unter Bildung von Monoxanthylderivaten um[1], während zyklische Ureide, wie Parabansäure[2] und 5,5-disubstituierte Barbitursäuren[3], Dixanthylderivate liefern. Phthalylharnstoff

[1] A. PIUTTI, Liebigs Ann. Chem. *214*, 17 (1882). – C. S. SMITH und C. J. CAVALLITO, J. Amer. Chem. Soc. *61*, 2218 (1939).

[2] T. W. EVANS und W. M. DEHN, J. Amer. Chem. Soc. *51*, 3651 (1929).

[3] G. HELLER und P. JACOBSOHN, Ber. dtsch. chem. Ges. *54*, 1107 (1921).

[1] R. FOSSE, C. r. Acad. Sci. *145*, 813 (1907); *158*, 1432 (1914). – W. ADRIANI, Rec. Trav. chim. *35*, 180 (1915).

[2] Vgl. R. FOSSE, *L'Urée* (Presses Universitaires de France, Paris 1928), S. 261.

[3] R. FABRE, J. Pharm. Chim. [7] *26*, 241 (1922); Bull. Soc. chim. France [4] *33*, 791 (1923).