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cis–trans Isomerization and Spectral Characteristics of Carotenoids and Some Related Compounds

BY L. ZECHMEISTER AND A. POLGÁR

After Gillam's discovery of the first isomerization phenomena in the field of C_{40}-carotenoids[1] and our interpretation of the reactions as spontaneous *trans–cis* rearrangements,[2] it became evident that the bending of the polyene molecule strongly affects both adsorption affinity and color. While, however, the adsorption forces may either be strengthened or weakened by these stereochemical changes,[2] the spectral alteration follows a uniform direction in every case, namely, the color of the solution or adsorbate becomes noticeably paler. The observations available at the present time are all in accordance with the results of a theoretical treatment given by Pauling.[3] According to Pauling the hydrogen atoms of CH-groups adjacent to a double bond with a coplanar *cis* configuration overlap somewhat and this has two significant effects: it decreases the stability of the molecule, and it shifts the extinction maxima to shorter wave lengths.

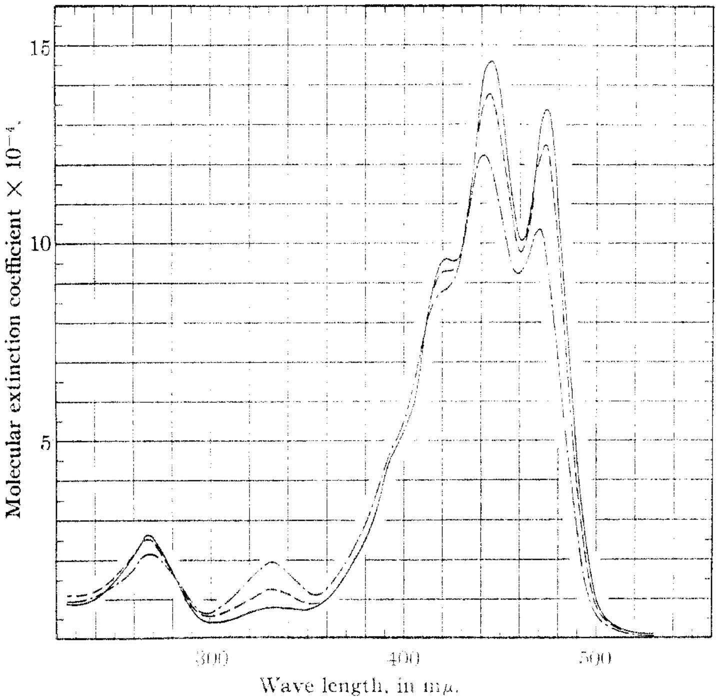

Fig. 1.—Molecular extinction curves of α-carotene in hexane: —— fresh solution of the all-*trans* compound, - - - mixture of stereoisomers after refluxing, and - · - after iodine catalysis.

While a reading in the visual spectroscope offers the possibility of providing some information on the stereochemical situation of a carotenoid, especially if it is repeated after the formation of a stereochemical equilibrium mixture by the addition of some iodine, a deeper insight can be expected from the comparative study of extinction curves of all-*trans* pigments and their stereoisomers. Spectral curves of fresh extracts or solutions of some carotenoids and of solutions partially isomerized by heat have been given by Strain,[4] by Beadle and Zscheile,[5] and by White, Zscheile and Brunson.[6] Their readings extend down to about 380 mμ. It has been customary in our Laboratory for some time to characterize each natural or synthetic polyene under investigation by three molecular extinction curves covering the spectral region reaching down to 220 mμ: first, a fresh pigment solution is examined, then, second, the same solution after forty-five minutes of refluxing, and, third, another portion after an hour of standing at room temperature in the presence of iodine (in amount 2% of the pigment). Such curves, taken in a Beckman spectrophotometer, are given in Figs. 1 to 3 and 6 to 7.

As a consequence of *trans–cis* changes both the heights and the wave lengths of the visible maxima and the well-known maxima in the far ultraviolet decreased in each case. Moreover, an unexpected phenomenon has appeared in a cer-

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(1) A. E. Gillam and M. S. El Ridi, *Biochem. J.*, **29**, 2465 (1935), and **30**, 1735 (1936).

(2) L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1350 (1939); L. Zechmeister, L. Cholnoky and A. Polgár, *ibid.*, **72**, 1678, 2039 (1939).

(3) L. Pauling, *Fortschr. Chem. organ. Naturstoffe*, **3**, 203 (1939), *cf.* footnote 9.

(4) H. H. Strain, "Leaf Xanthophylls," Washington, 1938: see *e. g.*, Fig. 18, p. 108; *J. Biol. Chem.*, **127**, 191 (1938).

(5) B. W. Beadle and F. P. Zscheile, *ibid.*, **144**, 21 (1942).

(6) J. W. White, F. P. Zscheile and A. M. Brunson, THIS JOURNAL, **64**, 2603 (1942); J. W. White, A. M. Brunson and F. P. Zscheile, *Ind. Eng. Chem., Anal. Ed.*, **14**, 798 (1942).

tain ultraviolet region, that between 320 and 380 mμ. Each C_{40}-carotenoid investigated so far, whether belonging to the type of hydrocarbons, alcohols, or hydroxy ketones, developed on stereoisomerization a moderate but marked maximum somewhere within this region, in which the curve of the corresponding all-*trans* compound showed only a very slight elevation. The maximum was found to be higher after iodine catalysis than after refluxing, showing the existence of two different equilibria.

This phenomenon, termed briefly the "*cis*-peak" effect, is characterized by the values contained in Table I. The table shows that for C_{40}-carotenoids the *cis*-peak has a definite location; the difference between its wave length and that of the longest wave length maximum in the visible region of the all-*trans* form is practically a constant, *viz.*, 142 mμ (±2mμ). The relative position of the two maxima mentioned is practically not influenced by the presence of functional groups.

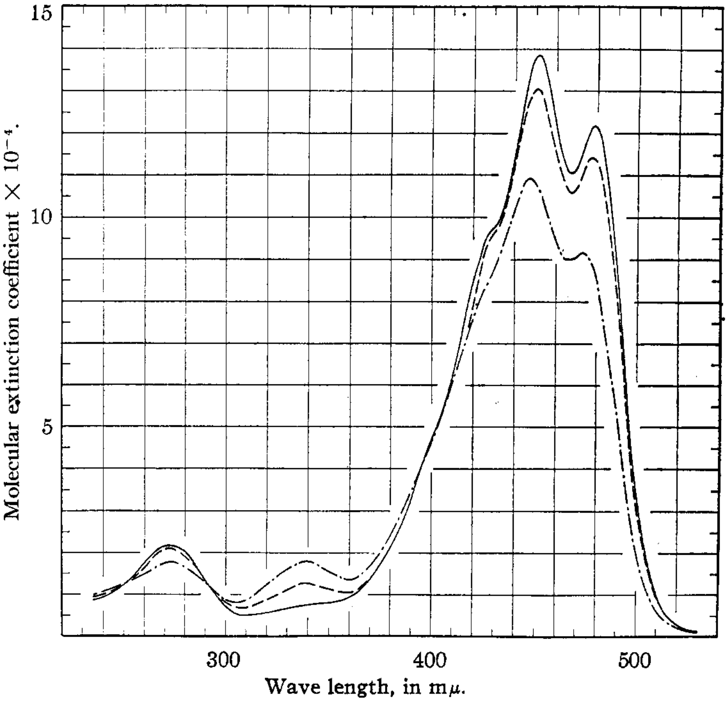

Fig. 2.—Molecular extinction curves of β-carotene in hexane: —— fresh solution of the all-*trans* compound, - - - mixture of stereoisomers after refluxing, and —·— after iodine catalysis.

With reference to some other types of polyenes the following remarks may suffice. The spectral curve of the lower molecular weight carotenoid, methylbixin, $C_{22}H_{26}(COOCH_3)_2$, shows some peculiarities and a *cis*-peak at 351 mμ which is 138 mμ shorter in wave length than the longest wave length maximum of "stable" methylbixin.[7] Diphenyloctatetraene, $C_6H_5(CH{=}CH)_4C_6H_5$, develops a *cis*-peak at 283 mμ when treated with iodine or refluxed, the wave length difference being only 112 mμ in this case.[8] Vitamin A is under spectrophotometric and chromatographic investigation. It possesses only two stereochemically available double bonds,[9] and the number of possible stereoisomers is four, the ordinary all-*trans* form included. The well-known extinction maximum near 328 mμ (in hexane) is decreased in height by a catalytic treatment with iodine at room temperature, and a simultaneous increase in the extinction appears in the region 240–280 mμ. No definite peak was observed in this case. It remains to be shown how far conversions other than the stereoisomerization of the vitamin may be responsible for the change in the extinction as represented in Fig. 8.

TABLE I

POSITION OF THE "*cis*-PEAK" IN THE SPECTRAL CURVE OF STEREOISOMERIC EQUILIBRIUM MIXTURES OF C_{40}-CAROTENOIDS OBTAINED BY IODINE CATALYSIS

Polyene	Position of the *cis*-peak in hexane (mμ)	Distance between *cis*-peak and longest wave length maximum of the all-*trans* form (mμ)
5,6-Dihydro-α-carotene[a]	328	141
5,6-Dihydro-β-carotene[a]	331	143
α-Carotene	331	143
Lutein[b]	331	143
β-Carotene	339.5	141
Cryptoxanthin	339	141
Physalien	338	141
γ-Carotene	349	143
Gazaniaxanthin[c]	349	142
Lycopene	361	141
Capsanthin	354	144
Celaxanthin[d]	380	144

[a] A. Polgár and L. Zechmeister, THIS JOURNAL, **65**, 1528 (1943). [b] *Ex Helianthus annuus.* [c] L. Zechmeister and W. A. Schroeder, THIS JOURNAL, **65**, 1535 (1943). [d] A. L. LeRosen and L. Zechmeister, *Arch. Biochem.*, **1**, 17 (1942).

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(7) From an unpublished investigation in collaboration with Mr. R. B. Escue; *cf.* L. Zechmeister and R. B. Escue, *Science*, **96**, 229 (1942).

(8) From an unpublished investigation in collaboration with Dr. A. L. LeRosen; *cf.* L. Zechmeister and A. L. LeRosen, THIS JOURNAL, **64**, 2755 (1942).

(9) *Cf.* L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Natl. Acad. Sci.*, **27**, 468 (1941).

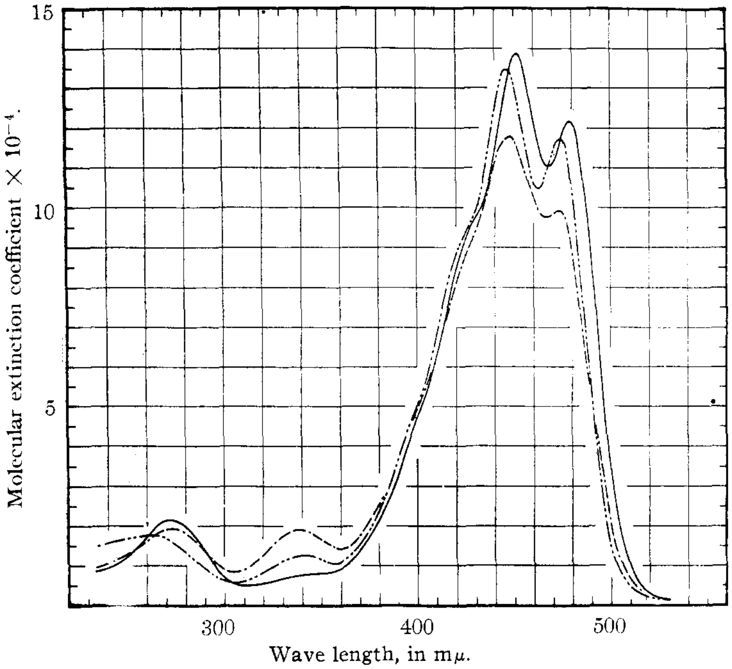

Fig. 3.—Molecular extinction curves of neo-β-carotene U, compared with that of β-carotene in hexane: —— β-carotene, –··– fresh solution of neo-β-carotene U, and ----- mixture of stereoisomers obtained from neo U by iodine catalysis.

Following the spectrophotometric analysis of the egg yolk pigment by a photoelectric method by Kuhn and Smakula,[10] many extinction curves of C_{40}-carotenoids extending into the ultraviolet have been published, a complete enumeration of which is not intended.[11] The reason why the *cis*-peak effect has not been found and interpreted as an isomerization phenomenon earlier is partially explained by the circumstance that some investigators, for example Karrer and Rüegger,[12] who measured extinctions in the whole range reaching down to 200 mμ did not study the spectral influence of stereoisomerization, while some papers mentioned above,[4,5,6] in which curves can be found, do not refer to wave length regions below 380 mμ. In the remarkable article of Smakula[13] the curves given for "isomethylbixin" and "methylbixin" show a difference which may well correspond to a *cis*-peak effect.

Miller[14] investigated ultraviolet spectra of β-carotene and lycopene, in alcohol–ether, both in fresh solutions and after standing in air for forty-eight to one hundred and twenty hours. He observed the appearance of a new maximum at 340 mμ for β-carotene and at 366 mμ for lycopene. The former figure corresponds exactly with the position of our *cis*-peak (in hexane). Miller ascribed the effect to oxidation which was the only reasonable interpretation six years ago when the stereoisomerization of carotenoids was still unexplored. The same statement is valid with reference to an earlier paper of McNicholas.[14a]

Subsequently Gillam and his associates[15] published spectral curves of several naturally occurring carotenoids in chloroform solution. These curves, which also appear in Morton's recent

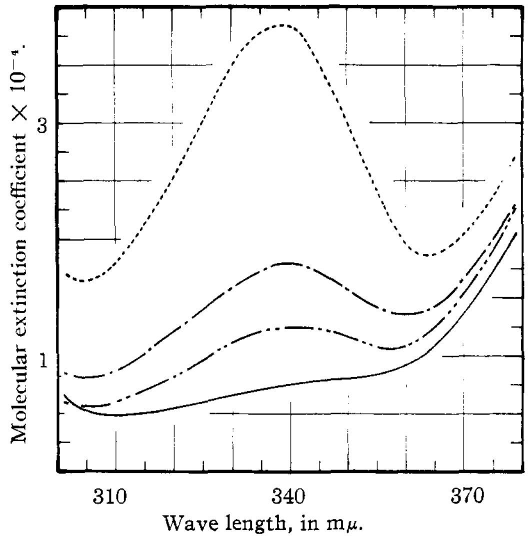

Fig. 4.—Molecular extinction curves of some β-carotene stereoisomers in the *cis*-peak region (in hexane); —— all-*trans*-β-carotene, ··—·· neo U, ···· mixture of isomers adsorbed below the all-*trans* form, and —·—· iodine equilibrium mixture.

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[10] R. Kuhn and A. Smakula, *Z. physiol. Chem.*, **197**, 161 (1939).

[11] *Cf.* the general reviews: E. R. H. Jones, *Ann. Rep. Progr. Chem.*, **37**, 290 (1940); G. Mackinney, *Ann. Rev. Biochem.*, **9**, 459 (1940); F. P. Zscheile, *Botan. Rev.*, **7**, 587 (1941); O. H. Walker, "Absorption Spectrophotometry and its Applications, Bibliography and Abstracts 1932 to 1938," A. Hilger Ltd., London, 1939. For a theoretical treatment of connected problems see L. Pauling, *Proc. Natl. Acad. Sci.*, **25**, 577 (1939); G. N. Lewis and M. Calvin, *Chem. Rev.*, **25**, 273 (1939); R. S. Mulliken, *J. Chem. Phys.*, **7**, 121, 364, 570 (1939). *Cf.* K. W. Hausser, R. Kuhn, A. Smakula and K. H. Kreuchen, *Z. physik. Chem.*, **B29**, 363 (1935), and the subsequent papers on pp. 371, 378, 384, 391 and 417, furthermore, R. S. Mulliken, *Rev. Modern Phys.*, **14**, 265 (1942).

[12] P. Karrer and A. Rüegger, *Helv. chim. acta*, **23**, 944 (1940).

[13] A. Smakula, *Angew. Chem.*, **47**, 657 (1934).

[14] E. S. Miller, *Plant Physiol.*, **12**, 667 (1937). The curves are reproduced in E. S. Miller, "Quantitative Biological Spectroscopy," Burgess Publishing Co., Minneapolis, Minn., 1940, p. 146, fig. 12.

[14a] H. J. McNicholas, *Bureau Stand. J. Res.*, **7**, 171 (1931).

[15] A. E. Gillam, I. M. Heilbron, R. A. Morton, G. Bishop and J. C. Drummond, *Biochem. J.*, **27**, 878 (1933); A. E. Gillam, *ibid.*, **29**, 1831 (1935).

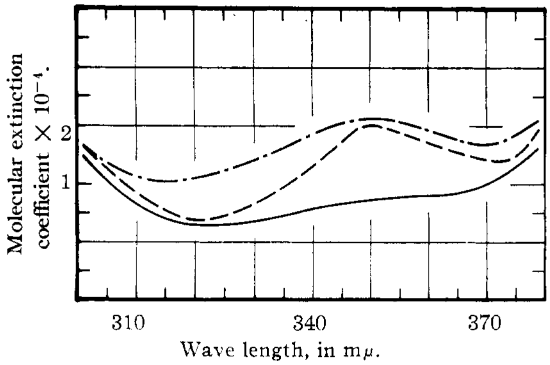

Fig. 5.—Molecular extinction curves of β-carotene in chloroform: ——— fresh solution of the all-*trans* compound, —·— after iodine catalysis, and – – – the corresponding section of the curve published by Gillam, *et al.*,[15] for butter carotene.

monograph,[16] show marked maxima in the region expected for the case of a *trans–cis* isomerization. We repeated the investigation of this region by using fresh solution of β-carotene in pure, acid-free chloroform. As demonstrated by Fig. 5, the new curve does not contain the maximum (at about 350 mμ) given by the authors mentioned. A corresponding peak developed, however, on addition of iodine.

In our Laboratory mainly stereochemical mixtures have been investigated so far. As a next step the contribution of individual stereoisomers to the *cis*-peak effect, after adequate separation in the Tswett column, is now being studied. In such experiments the amount of non-crystallizable isomers also can be estimated photometrically *e. g.*, after the conversion into the equilibrium mixture with iodine.

Some information is available concerning β-carotene, one of whose crystallizable stereoisomers, neo-β-carotene U, containing one or two *cis*-bonds, has been described recently.[17] As shown by chromatography, the iodine equilibrium mixture obtained from β-carotene consists of about 50% unchanged all-*trans* compound, 20% neo U, and 30% other stereoisomers. Fresh solutions of neo U crystals showed a considerably lower *cis*-peak than that of the total iodine equilibrium mixture in corresponding molar concentration (Fig. 3). Consequently, the 30% fraction must contain such stereoisomers whose *cis*-peak is much higher than that observed on addition of iodine to β-carotene solutions. This assumption was confirmed by the experiment (Fig. 4).

We do not claim that generally the height of a *cis*-peak must be proportional to the number of *cis*-double bonds present, considering the probable dependence of the contribution on the position of each double bond in the long polyenic chain. Furthermore, it remains to be shown how far the phenomenon is affected by the presence of a large number of *cis*-bonds. Experiments which are being made in collaboration with Dr. A. L. LeRosen and Dr. W. A. Schroeder, on the spectral curves of the poly-*cis* compounds prolycopene[18] and pro-γ-carotene[19] may clarify this point.

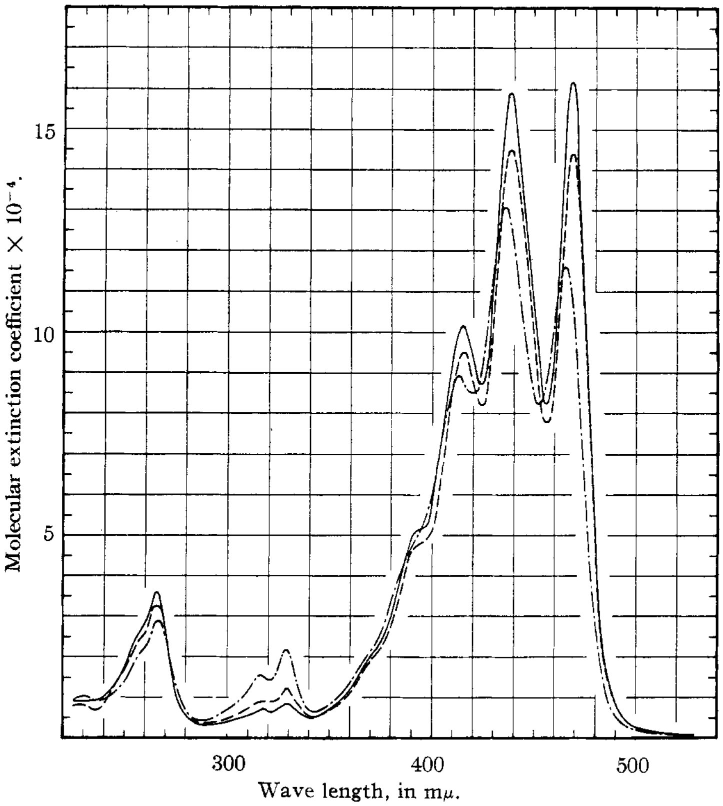

Fig. 6.—Molecular extinction curves of 5,6-dihydro-α-carotene in hexane: —— fresh solution of the all-*trans* compound, – – – mixture of stereoisomers after refluxing, and —·— after iodine catalysis.

In summarizing we can say that the general

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[16] R. A. Morton, "The Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes," A. Hilger Ltd., London, 2nd ed., 1942.

[17] A. Polgár and L. Zechmeister, This Journal, **64**, 1856 (1942).

[18] A. L. LeRosen and L. Zechmeister, *ibid.*, **64**, 1075 (1942), and Footnote 9.

[19] L. Zechmeister and W. A. Schroeder, *ibid.*, **64**, 1173 (1942).

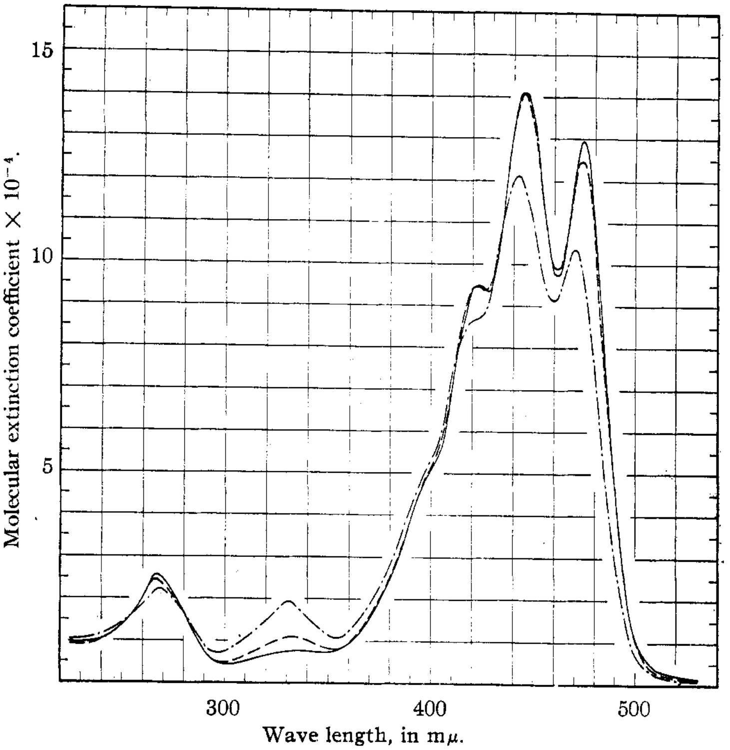

Fig. 7.—Molecular extinction curves of 5,6-dihydro-β-carotene in hexane: —— fresh solution of the all-*trans* compound, - - - mixture of stereoisomers after refluxing, and —·— after iodine catalysis.

shape of the spectral curves of carotenoids in the visible region is relatively little affected by *trans-cis* rearrangements; the presence of the usual two or three maxima which can be observed visually is evidently connected with the very presence of the long conjugated system.[20] In contrast, the stereochemical situation seems to find a more specific expression in a certain region of the ultraviolet.

Experimental

Materials and Apparatus.—"Hexane" (from petroleum, practical, Eastman Kodak Co.) was purified by shaking with fuming sulfuric acid and with alkaline permanganate, and rectified over permanganate crystals. The fraction between 62–65° was used for spectrophotometric purposes. In isomerization experiments involving refluxing, the blank to be used in the photometer was refluxed in the same manner as the pigment solution. When chloroform was used as a solvent, it was washed with sodium bicarbonate, sodium thiosulfate, and water immediately before the spectrophotometric experiment.

Chromatographically homogeneous samples of α-carotene (m. p. 183°, cor.) and β-carotene (m. p. 179.5°, cor.) were prepared from commercial crystalline carotene, obtained from S. M. A. Corp., Chagrin Falls, Ohio, and Barnett Laboratories, Long Beach, California. In some experiments β-carotene isolated from silk oak flowers[21] was used. The preparation of neo-β-carotene U has been described recently.[17] The preparation and analytical data of 5,6-dihydro-α-carotene and 5,6-dihydro-β-carotene are contained in the following paper.[22]

Anal. Calcd. for $C_{40}H_{56}$: C, 89.48; H, 10.52. Found (α-carotene): C, 89.32; H, 10.49; (β-carotene) C, 89.56; H, 10.20; and (neo-β-carotene U) C, 89.46; H, 10.52. $[\alpha]^{25}_{Cd} = +(100 \times 0.46°):(2 \times 0.064) = +359°$ (α-carotene in benzene).

In the experiments with vitamin A the product "Vitamin A alcohol, crystallized" (Eastman Kodak Co.) was used, the molecular extinction coefficient of which at 328 mμ agreed with that reported in the literature.[23]

The quantitative readings were taken with a Beckman photoelectric spectrophotometer.[24]

Methods.—In most cases about 2 mg. of substance was weighed on a semimicro balance and dissolved in 50 ml. of cold hexane. Three 5-ml. portions were used for the three curves needed (that of a fresh solution, of a refluxed solution, and of a solution catalyzed with iodine). The first portion was diluted tenfold (7 to 8 × 10^{-6} molar), the second was refluxed in a carbon dioxide atmosphere for forty-five minutes, after eightfold dilution; its volume was then made up to 50 ml. To the third portion 5 ml. of an iodine solution (containing 1 μg. of iodine per ml.) was added, and the total volume made up to 50 ml. This solution was allowed to stand at 23–25° for an hour before the photometric investigation. A mixture of 45 ml. of hexane

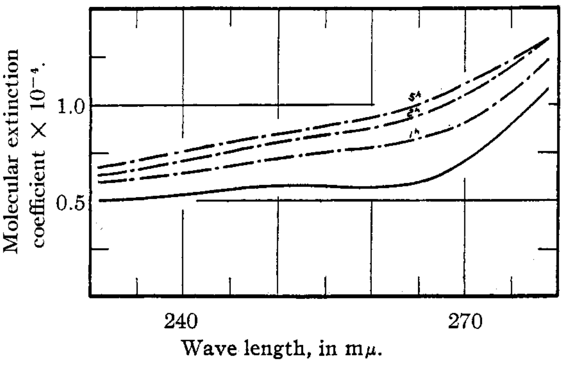

Fig. 8.—Molecular extinction coefficients of vitamin A in hexane between 230 mμ and 280 mμ: —— fresh solution of crystalline vitamin A (the readings were concluded within 21 min. after dissolution), —·— one, two and five hours after the addition of iodine (the five-hour curve remained practically unaltered after seven hours).

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(20) *Cf.*, *e. g.*, G. N. Lewis and M. Calvin, Footnote 11.

(21) L. Zechmeister and A. Polgár, *J. Biol. Chem.*, **140**, 1 (1941).

(22) A. Polgár and L. Zechmeister, This Journal, **65**, 1528 (1943).

(23) J. G. Baxter and C. D. Robeson, *ibid.*, **64**, 2411 (1942).

(24) H. H. Cary and A. O. Beckman, *J. Opt. Soc. Amer.*, **31**, 682 (1941).

and 5 ml. of the iodine solution served as a blank in this case.

In most cases two to four sets of values were obtained for each curve, using independent samples and taking readings at 1 to 5 mμ intervals in the course of an hour. For the final curves (Figs. 1–8) average values were plotted (Tables II and III). The greatest deviation of single points at the main maxima was ±1.3% for homogeneous all-*trans* compounds, ±1.9% for neo-β-carotene U, and ±2.5% for stereoisomeric mixtures (however, ±4.3% in the iodine curves of 5,6-dihydro-α-carotene). The molecular extinction coefficients were calculated in the usual manner[25]

$$E^{\text{mol.}}_{\text{1cm.}} = \frac{1}{L.c} \cdot \log \frac{I_0}{I}$$

where L = thickness of the stratum, c = concentration (moles per liter).

TABLE II

MOLECULAR EXTINCTION COEFFICIENTS OF α-CAROTENE, β-CAROTENE, AND NEO-β-CAROTENE U, AND OF THEIR STEREOISOMERIC EQUILIBRIA AT THE MAXIMA AND MINIMA IN HEXANE[a]

Fresh solution		After heat isomerization		After iodine isomerization	
mμ	$E^{\text{mol.}}_{\text{1cm.}} \times 10^{-4}$	mμ	$E^{\text{mol.}}_{\text{1cm.}} \times 10^{-4}$	mμ	$E^{\text{mol.}}_{\text{1cm.}} \times 10^{-4}$
		α-Carotene			
474	*13.4*	*474*	*12.5*	*470*	*10.4*
461	10.1	461	9.8	458–9	9.3
445–6	*14.6*	*444*	*13.8*	*441*	*12.2*
424–7	9.6	424[b]	9.3	421[b]	8.8
422	*9.6*				
344–8	0.7(4)	352	0.8(7)	353	1.14
333–4	*0.8(1)*	*331–2*	*1.27*	*331*	*1.96*
300–3	0.4(0)	298–9	0.5(6)	297–8	0.6(5)
267	*2.64*	*268*	*2.54*	*268*	*2.19*
		β-Carotene			
478	*12.2*	*477–9*	*11.4*	*473*	*9.2*
468	11.1	467	10.6	466	9.0
452	*13.9*	*451*	*13.1*	*447*	*10.9*
429[b]	9.7	429[b]	9.4	426[b]	8.4
348[b]	0.8(0)	358	1.04	360–1	1.36
		339–40	*1.27*	*339–40*	*1.80*
310	0.4(9)	308	0.6(7)	305–6	0.8(1)
272	*2.17*	*272*	*2.10*	*273*	*1.78*
		Neo-β-carotene U			
474	*11.7*	*473–5*	*11.2*	*473–4*	*9.9*
463	10.5	462–4	10.2	466–9	9.8
446–7	*13.5*	*445–7*	*13.1*	*447–9*	*11.8*
426[b]	9.5	426[b]	9.3	425[b]	8.7
358	1.06	358	1.23	359–62	1.42
342–3	*1.25*	*342–3*	*1.48*	*338–40*	*1.92*
306	0.5(6)	304	0.6(0)	305	0.8(1)
265	*1.76*	*265–6*	*1.73*	*271–4*	*1.93*

[a] The values of the maxima are italicized. [b] Point of inflection.

TABLE III

MOLECULAR EXTINCTION COEFFICIENTS OF 5,6-DIHYDRO-α-CAROTENE, 5,6-DIHYDRO-β-CAROTENE, AND OF THEIR STEREOISOMERIC EQUILIBRIA AT THE MAXIMA AND MINIMA IN HEXANE[a]

Fresh solution		After heat isomerization		After iodine isomerization	
mμ	$E^{\text{mol.}}_{\text{1cm.}} \times 10^{-4}$	mμ	$E^{\text{mol.}}_{\text{1cm.}} \times 10^{-4}$	mμ	$E^{\text{mol.}}_{\text{1cm.}} \times 10^{-4}$
		5,6-Dihydro-α-carotene			
469	*16.2*	*469*	*14.4*	*466*	*11.6*
456	8.2	456	7.8	452	8.2
438	*15.9*	*438–9*	*14.5*	*436*	*13.1*
424	8.7	424–5	8.2	420–1	8.5
415	*10.2*	*415–6*	*9.5*	*413*	*8.9*
395[b]	5.2	395[b]	4.8		
340	0.4(9)	342–3	0.5(2)	342–3	0.6(7)
329	*0.8(3)*	*329*	*1.22*	*329*	*2.17*
321	0.6(5)	322	0.8(9)	320–1	1.42
318	*0.7(2)*	*318*	*0.9(1)*	*316*	*1.55*
288–9	0.3(3)	288–92	0.3(8)	288–9	0.4(3)
266	*3.6*	*266*	*3.4*	*266–7*	*2.88*
		5,6-Dihydro-β-carotene			
474–5	*12.9*	*473*	*12.5*	*471*	*10.4*
462	9.7	462	9.9	460	9.1
445	*14.1*	*445*	*14.1*	*443*	*12.1*
428	9.4	425	9.4	421[b]	8.7
422–5	*9.5*	*423*	*9.5*		
348–9	0.7(2)	354	0.8(1)	354	1.07
334–6	*0.7(7)*	*333*	*1.12*	*331*	*1.94*
302	0.4(3)	298–300	0.4(8)	297	0.7(2)
267	*2.58*	*266–7*	*2.46*	*268*	*2.23*

[a] The values of the maxima are italicized. [b] Point of inflection.

Treatment of Non-crystallizable Stereoisomers.—Since any individual component (or a mixture of components) of a certain stereoisomeric equilibrium gives identical extinction values on iodine catalysis, its concentration can easily be determined if the iodine equilibrium curve is known. In the present investigation it was convenient to use catalyzed all-*trans* solutions for determining the iodine curve but in principle any crystallizable stereoisomer belonging to the equilibrium may be used.

In order to establish the contribution of the non-crystallizable isomers of β-carotene to the *cis*-peak of the total stereoisomeric mixture (Fig. 4), a catalyzed solution of 2 mg. of β-carotene in hexane was developed with the same solvent on a lime column (17 × 1.9 cm.; Shell Brand lime, chemical hydrate, 98% through 325 mesh).[26] The column was then extruded and the zones containing unchanged β-carotene, neo-β-carotene U, as well as a minor top layer were discarded. All remaining colored zones were combined, eluted rapidly with alcohol, transferred into hexane, washed free of alcohol and dried. One-half of this solution was investigated in the spectrophotometer immediately, the other half after the addition of iodine and standing for an hour. The concentration derived from the latter readings was used for the calculation of the molecular extinctions. The height of the *cis*-peak of this fraction (Fig.

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(25) We suggest for the molecular extinction coefficient the use of $E^{\text{mol.}}_{\text{1cm.}}$ instead of ε or κ, in accordance with the widely accepted symbol $E^{1\%}_{\text{1cm.}}$.

(26) Since commercial lime contains impurities showing strong extinction in the region below 330 mμ, it was necessary to pre-treat the column by sucking considerable amounts of a petroleum ether–acetone mixture (1:1) through it. The column was washed with petroleum ether and then with pure hexane before the chromatography of the catalyzed solution was begun.

4, dotted line) established as just described was identical with a value calculated from the *cis*-peak of the iodine equilibrium mixture after deduction of the contributions of β-carotene and its U-isomer.

Summary

While the wave lengths and intensities of the main maxima of carotenoids are decreased by *trans-cis* shifts, there develops in the ultraviolet region, between 320 and 380 mμ a new marked maximum, when a hexane solution is refluxed or catalyzed with iodine. This phenomenon is termed the "*cis*-peak" effect. The absolute position of the new maximum in the spectral curve depends on the structure of the chromophoric system. Its distance from the highest wave length maximum of an all-*trans* C_{40}-carotenoid is practically a constant, *viz.*, 142 mμ.

Methylbixin and diphenyloctatetraene show qualitatively the same phenomenon. On addition of some iodine vitamin A shows an increase of its extinction in the region 240 to 280 mμ.

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Action of Cold Concentrated Hydriodic Acid on Carotenes: Structure and *cis-trans*-Isomerization of Some Reaction Products

BY A. POLGÁR AND L. ZECHMEISTER

The action of strong chemicals on carotenoids is a nearly unexplored field because it has been more or less tacitly assumed that the sensitive polyene chain is destroyed by such treatment. It was found, however, that when a petroleum ether solution of β-carotene is shaken with concentrated hydriodic acid under strictly defined conditions, not more than half of the pigment is converted into colorless or faintly colored cleavage products. If the reaction mixture is differentiated on the Tswett column, the chromatogram shows the absence of unchanged β-carotene and the presence of several yellow zones, two of which have been crystallized. Both compounds can be obtained either from β- or from α-carotene. They belong to the C_{40}-class of polyene hydrocarbons and the molecules terminate in two cyclic structures as demonstrated by the negative result of the isopropylidene estimations.

Of the two compounds that one which possesses stronger adsorption affinity for lime is a provitamin A and must contain an unsubstituted β-ionone ring. It is so strikingly similar to α-carotene that, at first, it was tentatively identified as the racemic form of the latter. The spectral bands as observed visually before and after the addition of iodine, and even the shape of the spectral curves in the visible and ultraviolet regions were congruent. Furthermore, the compound did not separate from natural α-carotene in the mixed chromatogram. This whole behavior clearly indicates the presence of a chromophoric group which is composed of ten conjugated double bonds. While, as is well-known, α-carotene contains an eleventh double bond in an isolated position, the catalytic hydrogenation of our compound in the presence of platinum oxide demonstrated a total number of only ten such bonds.

On the basis of the experimental evidence the compound must have the structure expressed below by the first formula; it is conveniently termed 5,6-dihydro-β-carotene. We suppose that, under the influence of the strong acid, one of the end double bonds of β-carotene has migrated out of conjugation and has been reduced. The same product is formed from α-carotene by saturation of its isolated double bond.

The second compound (which is adsorbed below the first one in the column) has also been obtained from both natural carotenes. As it does not display any provitamin A activity in the rat, it cannot contain a β-ionone ring. It is evident that both ends of the β-carotene molecule have been altered by the hydrogen iodide treatment. The spectral maxima of this compound are located at considerably shorter wave lengths than those of 5,6-dihydro-β-carotene and indicate the presence of nine conjugated double bonds. Since catalytic hydrogenation revealed the presence of a total of ten such bonds, one of them must be in an isolated position. For this compound we suggest the structure of 5,6-dihydro-α-carotene (see the second formula).

The formation of 5,6-dihydro-α-carotene from