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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[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 918]

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

BY A. POLGÁR AND L. ZECHMEISTER

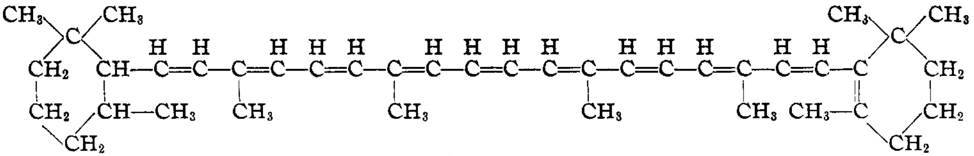
The action of strong chemicals on carotenoids is a nearly unexplored field because it has been more or less tacitly assumed that the sensitive polyene chain is destroyed by such treatment. It was found, however, that when a petroleum ether solution of β-carotene is shaken with concentrated hydriodic 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.

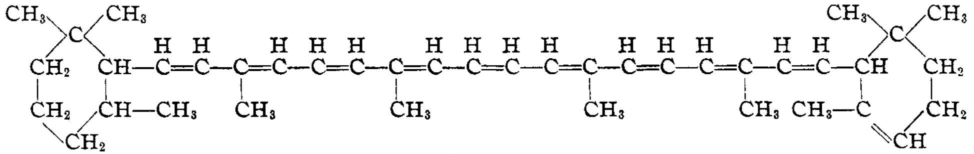
On the basis of the experimental evidence the compound must have the structure expressed below by the first formula; it is conveniently termed 5,6-dihydro-β-carotene. We suppose that, under the influence of the strong acid, one of the end double bonds of β-carotene has migrated out of conjugation and 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



5,6-Dihydro-β-carotene



5,6-Dihydro-α-carotene

β-carotene can be explained by the assumption that both end double bonds migrate out of conjugation but that only one of them is reduced under the conditions of the experiment. If α-carotene is treated with hydriodic acid, a double bond migration within the β-ring and the addition of two hydrogen atoms to the migrating double bond should be taken into consideration as a plausible reaction mechanism.

According to the formulas, the two dihydrocarotenes are related in a manner analogous to that in which α-carotene and β-carotene are related, *i. e.*, one cyclic system is identical in both compounds while the other is either an α- or a β-ionone ring.

The result of the hydriodic acid experiments is dependent on the concentration and quality of the acid. Diluted hydriodic acids do not give satisfactory results. If commercial concentrated acid (55–58%), containing much free iodine, is used, all the initial carotene disappears and the product isolated consists chiefly (in some cases exclusively) of 5,6-dihydro-α-carotene. When, however, most or all of the iodine is eliminated by means of mercury or hypophosphorous acid, the conversion assumes a markedly milder character. In such experiments some unchanged carotene appears in the chromatogram, and the yields of 5,6-dihydro-β-carotene are increased, partially at the expense of the corresponding α-derivative. In the most favorable case 100 parts of β-carotene gave nine parts of each of the two products.

The two stable, crystalline dihydro compounds discussed are entirely different from some dihydrocarotenoids formed by Thiele addition and described as oily substances and from a well-known crystalline compound.[1]

Because of their polyenic structure, 5,6-dihydro-α- and -β-carotenes are subject to reversible *trans–cis* isomerization under the influence of refluxing, iodine catalysis or melting. Of the 32 possible stereoisomers[2] six have been observed in the case of the β-derivative and six of the α-derivative (Table I). As given in the Experimental Part some of the stereoisomers formed are adsorbed below and others above the respective all-*trans* compound in the Tswett column. Consequently, our earlier assumption,[3] made on the basis of a first survey, that neohydrocarbons generally show weaker adsorbability than the corresponding all-*trans* forms, must now be modified.[4]

When all-*trans*-dihydro-α-carotene or -dihydro-β-carotene is converted into a stereochemical

TABLE I

VISUALLY DETERMINED SPECTRAL MAXIMA OF REVERSIBLY FORMED STEREOISOMERS OF 5,6-DIHYDRO-β-CAROTENE AND 5,6-DIHYDRO-α-CAROTENE, IN THE SEQUENCE OF DECREASING ADSORPTION AFFINITIES, IN PETROLEUM ETHER (B. P. 60–70°)

Name	mμ	
	5,6-Dihydro-β-carotene	
Neo U	470.5	443.5
Neo V	466	438
All-*trans*	477.5	447.5
Neo A	470	440.5
Neo B	466.5	437.5
Neo C	462.5	434
	5,6-Dihydro-α-carotene	
Neo T	459.5	430.5
Neo U	465	427
Neo V	458	431
All-*trans*	470.5	442.5
Neo A	462	435.5
Neo B	455	426.5

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(1) *Cf.*, *e. g.*, J. H. C. Smith, *J. Biol. Chem.*, **90**, 597 (1931); P. Karrer and R. Morf, *Helv. Chim. Acta*, **14**, 1033 (1931); R. Kuhn and H. Brockmann, *Z. physiol. Chem.*, **213**, 1 (1932); P. Karrer and A. Rüegger, *Helv. Chim. Acta*, **23**, 955 (1940).

(2) L. Pauling, *Fortschr. Chem. organ. Naturstoffe*, **3**, 203 (1939); L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Natl. Acad. Sci.*, **27**, 468 (1941).

(3) L. Zechmeister, L. Cholnoky and A. Polgár, *Ber.*, **72**, 1678 (1939).

(4) *Cf.* A. Polgár and L. Zechmeister, THIS JOURNAL, **64**, 1856 (1942).

equilibrium mixture, a spectroscopic "*cis*-peak" appears at 328 mμ and 331 mμ, respectively. The extinction curves were given in the foregoing paper.[5]

Acknowledgment.—The authors are indebted to Professor A. J. Haagen-Smit and Dr. G. Oppenheimer for microanalyses and especially for catalytic hydrogenations which were carried out in the micro-apparatus devised by Prater and Haagen-Smit.[6] The isopropylidene estimations were kindly carried out by Mr. W. A. Schroeder and the rat tests by Dr. C. E. P. Jeffreys.

Experimental

Materials and Apparatus.—The petroleum ether used (Skellysolve B) had a boiling range of 60–70°. The acid employed was Acid Hydriodic Merck Reagent, spec. gravity 1.70, containing 55–58% hydriodic acid and free iodine. The chromatographic tubes were those described earlier.[7] The absorbent was calcium hydroxide (Shell Brand lime, chemical hydrate; 98% through 325 mesh). Sintered glass funnels were used for elutions and the eluates were washed alcohol-free in a continuous apparatus.[8] Melting points (cor.) were taken in an electrically heated Berl block, in sealed capillary tubes filled with carbon dioxide. The spectra were determined in an Evaluating Grating Spectroscope as designed by Loewe and Schumm (Zeiss, light filter BG7). After the position of the maxima had been determined 1 to 2 drops of iodine solution (2–5 μg.) were added to the spectroscopic cell and the positions redetermined after a few minutes when constancy of the new values had been attained. The data for wave lengths given refer to petroleum ether solutions unless otherwise indicated. For the determination of the concentrations and ratios of pigments a Pulfrich Gradation Photometer was used (light filter S45 or S47).

Isolation of 5,6-Dihydro-α- and -β-carotene

(a) **From β-Carotene.**—The solution of 15 mg. of β-carotene in 180 ml. of petroleum ether was mechanically shaken with 30 ml. of concd. hydriodic acid for thirty minutes. The pigment solution when washed acid free and dried with sodium sulfate, showed about one-third of the original color intensity. It was developed on a lime column (26 × 5.8 cm.) with petroleum ether (the figures on the left side denote the width of the zones, in mm.)

1 orange } blurred section, contains at least two
2 faintly colored } crystallizable pigments
2 yellow } average maxima at 453, 424 mμ (no
10 yellow } change with iodine)
2 faintly colored
8 light yellow, unidentified: 446, 418.5 mμ (with iodine 450, 422 mμ)
7 faintly colored
17 orange, unidentified: 448.5, 420.5 mμ (447.5, 419 mμ)
5 colorless
45 dark orange, dihydro-β-carotene: 476, 444.5 mμ (474.5, 444 mμ)
35 light yellow, neos of the next zone: 464.5, 437 mμ (468.5, 438.5 mμ)
15 colorless
80 bright greenish-yellow, dihydro-α-carotene and stereoisomers: 468, 439 mμ (466.5, 437.5 mμ)

The zones of the two dihydro compounds (plus stereoisomers) were eluted separately with alcohol and transferred by water into petroleum ether which was dried and evaporated. Each of the powdery residues was dissolved in a minimum amount of cold benzene in a small centrifuge tube and crystallized by addition of excess methanol. After standing at 5° overnight the pigments were centrifuged, washed with methanol and recrystallized as described, in the same tube. The yield was approximately 1 mg. of each dihydro compound [m. p. 147°(β) and 201° (α), respectively].

In a larger scale experiment 72 ml. of concd. hydriodic acid was shaken first with 10 ml. of mercury for a few minutes; then 300 mg. of β-carotene in 750 ml. of petroleum ether was added and the shaking continued for fifteen more minutes. A dark, solid substance floated in the acid phase and dissolved upon addition of concd. sodium thiosulfate. After washing and drying a first chromatogram was developed with petroleum ether in a 4-liter percolator (36 × 16 × 9 cm.). The blurred sequence of pigments consisted of top layers (85 mm.), then of a section (53 mm.) containing some unchanged β-carotene among other pigments and finally of a lighter colored zone (70 mm.) which was well separated from the others. The eluate of this last section, combined with the yellow filtrate, was rechromatographed on two columns (30 × 8 cm.). The two dihydro compounds appeared as well separated main zones. After elution with alcohol and crystallization as described above, 18 mg. of 5,6-dihydro-β-carotene and 4.7 mg. of 5,6-dihydro-α-carotene were isolated. In the presence of hypophosphorous acid 500 mg. of β-carotene yielded 23.3 mg. of dihydro-β- and 19.5 mg. of dihydro-α-carotene.

(b) **From α-Carotene.**—A solution of 20 mg. of α-carotene in 250 ml. of petroleum ether gave the following chromatogram on treatment with 42 ml. of concd. hydriodic acid (column 25 × 5.8 cm.)

40 several irreversible, orange-yellow zones
25 faintly colored
27 dark orange, dihydro-β-carotene: 475, 455 mμ (with iodine 473, 444 mμ)
2 faintly colored
20 bright greenish-yellow, neo of the zone below: 465, 437.5 mμ (468, 441 mμ)
17 colorless
42 bright greenish-yellow, dihydro-α-carotene: 469, 439.5 mμ (467, 437 mμ)
25 weaker in color, neos of the above: 463, 434 mμ (466, 437.5 mμ)

The yields were 1.5 mg. of 5,6-dihydro-β-carotene (m. p. 153°) and 2.5 mg. of 5,6-dihydro-α-carotene (m. p. 195°). In the presence of hypophosphorous acid 110 mg. of α-carotene gave 4.3 mg. of its dihydro derivative.

(c) **From Commercial Carotene Mixture (90% β + 10% α), on Larger Scale.**—After shaking 1000 mg. of carotene in 1.3 liters of petroleum ether with 220 ml. of concd. hydriodic acid (containing iodine) for thirty minutes

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

(6) A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **12**, 704 (1940).

(7) L. Zechmeister and L. Cholnoky, "Principles and Practice of Chromatography," John Wiley and Sons, Inc., New York, N. Y., 1941, Fig. 19, facing p. 62. These tubes are manufactured by the Scientific Glass Apparatus Co., Bloomfield, New Jersey.

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

TABLE II

VISUALLY DETERMINED SPECTRAL MAXIMA OF 5,6-DIHYDRO-β-CAROTENE AND 5,6-DIHYDRO-α-CAROTENE SOLUTIONS

	5,6-Dihydro-β-carotene, mμ				5,6-Dihydro-α-carotene, mμ			
	without iodine		with iodine		without iodine		with iodine	
Carbon disulfide	509.5	476	506	473	501	486.5	499	464
Benzene	489	458	487.5	456	483.5	453.5	481	450.5
Chloroform	489	457	488	456	482.5	452.5	481	451
Petroleum ether (b. p. 86–100°)	478.5	449	478.5	448	472.5	444	471.5	443.5
Petroleum ether (b. p. 60–70°)	477.5	447.5	474.5	445	470.5	442.5	469.5	439.5
Hexane	475	445	472.5	442.5	469.5	441	467	437
Ethanol	477.5	448	477.5	448	471	443	471	443

at 25°, some dark, sticky material was floating in the aqueous phase. Upon addition of 1 vol. of water and some thiosulfate solution this precipitate disappeared. The lower phase was drained off and alcoholic thiosulfate was added to the upper one. After thorough washing and drying the pigment solution gave in a 6-liter percolator (46 × 20 × 9 cm.) a blurred chromatogram consisting of three heterogeneous pigment sections, 130 mm., 25 mm. and (near the bottom) 230 mm. wide, respectively. The alcohol eluate of the latter was transferred into petroleum ether and evaporated completely. The residue was dissolved in a minimum amount of cold benzene and crystallized with abs. methanol. After standing at 5° the crude crystals (213 mg.) were centrifuged, dissolved in 1 liter of petroleum ether and chromatographed on four columns (30 × 8 cm.). About four-fifths of the total adsorbed pigment was contained in a bright, slightly greenish-yellow zone of 5,6-dihydro-α-carotene which was accompanied by three of its stereoisomers, two above and one below the all-*trans*-zone. After further operations described in section (a) had been carried out, 107 mg. of 5,6-dihydro-α-carotene was isolated. The mother liquor yielded 15 mg. more (m. p. 202°).

A similar experiment carried out with 500 mg. of commercial carotene in the presence of mercury yielded 45 mg. of 5,6-dihydro-β-carotene and 61 mg. of 5,6-dihydro-α-carotene. On purification the latter yield decreased to 42 mg.

5,6-Dihydro-β-carotene.—When crystallized from carbon disulfide and ethanol or benzene (or chloroform) + methanol, characteristic leaf- and whetstone-like forms appear under the microscope (Fig. 1). Suspended plates show moderate glittering. Macroscopically, the compound forms a rather dark orange powder which, however, is lighter than β-carotene. The highest melting point observed was 164°; in some cases it was lower, *e. g.*, 155° and 160°. The solubility of the dihydro compound and its behavior in the partition test are very similar to that of α-carotene. The solutions did not show optical activity.

For analytical purposes three independent samples were used after drying in high vacuum at 56° for an hour. The first was free of ash; the figures for the other estimations are corrected for 0.5 and 1.3% ash, respectively.

Anal. Calcd. for $C_{40}H_{58}$: C, 89.15; H, 10.85; mol. wt., 539. Found: C, 89.02, 89.07, 88.69; H, 11.02, 10.87, 10.96; mol. wt. (in camphor), 540.

Catalytic Hydrogenation.—8.222 mg. in methylcyclohexane and glacial acetic acid (1:1) absorbed 3.72 ml. of hydrogen (over mercury, 23.5°, 742 mm.) in the presence of 21 mg. of PtO_2; 11.000 mg. with 22 mg. of PtO_2 absorbed 4.91 ml. (24°, 743 mm.). $C_{40}H_{58}$. Found: 9.8 and 9.7 double bonds. Control experiments gave 11.1 and 11.1 double bonds for β-carotene and 11.0(5) for α-carotene.

Isopropylidene Group.—On ozonolysis 8.92 mg. gave acetone corresponding to 0.53 ml. of $N/20$ iodine (after subtraction of the blank value). $C_{40}H_{58}$. Found: 0.27 $(CH_3)_2C=$. A control gave 0.29 "isopropylidene group" for β-carotene, 0.25 for α-carotene and 1.6 for lycopene.

For visual spectra see Table II, for spectral curves the foregoing paper.[5]

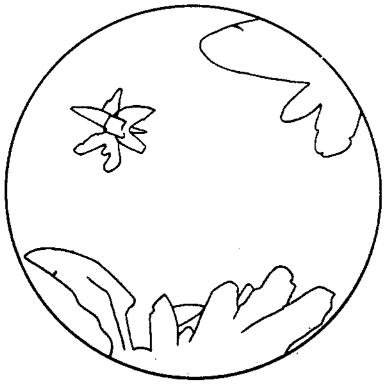

Fig. 1.—5,6-Dihydro-β-carotene, crystallized from carbon disulfide and ethanol.

For all practical purposes the concentrations of 5,6-dihydro-β-carotene solutions can be determined in the Pulfrich instrument (light filter S45) using the figures valid for α-carotene,[9] *viz.* (k = extinction coefficient, c = mg. of pigment in 100 ml. of petroleum ether)

k	0.2	0.4	0.6	0.8	1.0
c	0.082	0.172	0.264	0.356	0.454

On the lime chromatogram, developed with petroleum ether, the following sequence appears from top to bottom: β-carotene, α-carotene plus 5,6-dihydro-β-carotene, and 5,6-dihydro-α-carotene. On strong lime it is advisable to develop with petroleum ether containing 1–3% acetone in order to separate β-carotene from dihydro-β-carotene; in such cases dihydro-α-carotene is washed down into the chromatographic filtrate. For the separation of the two

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[9] These values were determined by L. Cholnoky.

dihydro compounds the use of petroleum ether is satisfactory; in all cases colorless interzones appear. For the separation of the stereoisomers of dihydro-β-carotene, developing with petroleum ether which contained 1% acetone was found more advantageous. It must be remarked that the section directly above the 5,6-dihydro-β-carotene zone contained a partially *cis* pigment (450, 424 mμ) and that it was difficult to obtain clean separation in this region.

cis–trans-Isomerization of 5,6-Dihydro-β-carotene

(a) On Standing or Refluxing.—A solution of 2.5 mg. of 5,6-dihydro-β-carotene in 15 ml. of petroleum ether was found practically unchanged after two hours' standing at 24°. On longer standing the extent of isomerization is considerably less than that of the α-isomer. The neo-compounds were adsorbed partially above and partially below the unchanged all-*trans* pigment in the Tswett column. The colorimetric ratio, unchanged all-*trans*: neo-forms (in petroleum ether) was found to be as follows

Hours of standing	2	29	68
Ratio	100:0	98:2	95:5

At 5° the ratio was 98:2 after sixty-eight hours.

A petroleum ether solution (50 ml.) containing 2.6 mg. of dihydro-β-carotene was refluxed in a carbon dioxide atmosphere for thirty minutes using an all-glass apparatus. After cooling with tap water, it was chromatographed (column, 20 × 3.8 cm.)

25 colorless
20 pale orange, neo U: 473, 443.5 mμ (with iodine 475.5, 444.5 mμ)
30 colorless
35 orange-yellow, unchanged all-*trans:* 477, 446.5 mμ (474, 444 mμ)
3 colorless
5 light yellow, neos B + C: 469.5, 439.5 mμ (474, 444 mμ)

Each zone was eluted with alcohol and transferred into petroleum ether. The colorimetric ratio was: Unchanged all-*trans*: neo U: neos B + C = 89:2:9. The B + C zone was combined with corresponding fractions from other experiments and rechromatographed. The spectra of these two difficultly separable stereoisomers were: neo B, 466.5, 437.5 mμ (with iodine 473.5, 443.5 mμ); neo C, 462.5, 434 mμ (472, 442.5 mμ). The adsorbate of the more prominent isomer B was bright yellow while the C-zone was paler. Possibly neo-5,6-dihydro-β-carotene A was also present in traces within the lowest section of the unchanged all-*trans* zone. This minor isomer can be separated with difficulty from the all-*trans* form, and was observed only when a larger amount of the pigment suffered isomerization (see below). An analogous statement is valid for the V-isomer mentioned below which may have been present in traces within the U-zone.

(b) Isomerization by Iodine Catalysis at Room Temperature.—A solution of 5 mg. of 5,6-dihydro-β-carotene in 50 ml. of petroleum ether was mixed with 0.1 mg. of iodine (in the same solvent), and chromatographed thirty minutes later (column, 23 × 4.8 cm.)

40 colorless
40 pale orange, neo U: 472, 442.5 mμ (with iodine 474.5, 444.5 mμ)
3 colorless
10 yellow, neo V: 467.5, 439 mμ (473.5, 444 mμ)
25 colorless
40 orange, unchanged all-*trans*: 476, 446 mμ (475, 446 mμ)
2 almost colorless
2 light brownish-orange, neo A: 472, 442 mμ (475, 446 mμ)
2 colorless
10 light yellow, neos B + C: 468, 437 mμ (474.5, 443.5 mμ)

The colorimetric ratio was: Unchanged all-*trans*: neo U:neo V:neo A:neos B + C = 67:12:6:3:12.

Each of the five solutions was catalyzed with iodine and chromatographed after thirty minutes of standing. The relative photometric values are listed in Table III.

TABLE III

RELATIVE PHOTOMETRIC VALUES OF 5,6-DIHYDRO-β-CAROTENE AND SOME STEREOISOMERS AS FORMED BY IODINE CATALYSIS OF THE INDIVIDUAL ISOMERS

Starting material	Relative photometric values (%)		
	Neo-forms with increased adsorbability (U + V)	all-*trans* form	Neo-forms with decreased adsorbability (A + B + C)
Neo U	17	63	20
Neo V	17	69	14
All-*trans*	18	67	15
Neo A	21	64	15
Neo B + C	16	70	14

(c) Isomerization with Hydriodic Acid and Hydrochloric Acid.—Because about half of the starting material undergoes an irreversible alteration, commercial concentrated hydriodic acid is not a suitable reagent for stereoisomerization experiments with dihydro-β-carotene. The formation of some 5,6-dihydro-α-carotene from the β-compound was observed in such experiments.

A solution of 3 mg. of 5,6-dihydro-β-carotene in 50 ml. of petroleum ether was shaken with 10 ml. of hydriodic acid (1 vol. of concd. acid + 4 vol. of water) for thirty minutes. The pigment solution was washed acid-free, dried with sodium sulfate and chromatographed (column, 20 × 3.8 cm.)

40 almost colorless
25 pale orange, neo U: 471, 443.5 mμ (with iodine, 474, 444.5 mμ)
3 colorless
25 faint yellow, neo V: 467, 439 mμ (474, 444 mμ)
5 almost colorless
55 orange-yellow, unchanged all-*trans*: 476, 445.5 mμ (474, 443.5 mμ)
2 colorless
8 light yellow, neos B + C: 471, 442 mμ (473.5, 443.5 mμ)

The colorimetric ratio, unchangeda ll-*trans*: neo U: neo V:neos B + C was 91:6:1:2.

In a parallel experiment commercial concentrated HCl was applied and the following chromatogram obtained

3 bright yellow (irreversible conversion product)
12 pale pink (irreversible conversion product)
15 colorless
17 pale orange, neo U: 471.5, 442.5 mμ (with iodine 474.5, 444.5 mμ)
1 colorless
10 faint yellow, neo V: 468.5, 440 mμ (474, 444.5 mμ)
5 colorless
40 orange-yellow, unchanged all-*trans*: 476, 445 mμ (474 443.5 mμ)
7 yellow, neo A: 472.5, 443 mμ (475, 444 mμ)
10 almost colorless
30 light yellow, neos B + C: 467.5, 439 mμ (474, 444 mμ)

The colorimetric ratio, unchanged all-*trans*: neo U:neo V:neo A:neos B + C was 67:13:2:5:13.

(d) **Isomerization by Melting.**—3.5 mg. of 5,6-dihydro-β-carotene crystals was melted in a sealed tube in carbon dioxide and kept in a bath at 170° for fifteen minutes. Only a moderate fraction was destroyed. After cooling in ice-water the petroleum ether solution of the melt was chromatographed (column, 20 × 3.8 cm.)

30 colorless
20 pale orange, neo U: 472, 442.5 mμ (with iodine 474, 444 mμ)
2 colorless
8 light yellow, neo V: 465.5, 437.5 mμ (474.5, 444 mμ)
15 colorless
35 orange-yellow, unchanged all-*trans*: 474, 444.5 mμ (474, 444 mμ)
13 yellow, neo A: 470.5, 441 mμ (473.5, 443 mμ)
3 colorless
30 light yellow, neos B + C: 466, 437 mμ (473.5, 442.5 mμ)

The colorimetric ratio, unchanged all-*trans*: neo U: neo V:neo A:neos B + C was 56:13:2:11:18.

5,6-Dihydro-α-carotene.—This polyene can be crystallized from carbon disulfide and alcohol and forms microscopic yellow or light yellow quadrangular plates with twinned and dented forms (Fig. 2). The crystals as obtained from benzene and methanol are not unlike those of natural α-carotene. The suspended crystals, when, *e. g.*, 0.1 mm. long, show glittering. Macroscopically, the powder is of dull, brick-like color, considerably lighter than α- or β-carotene. The melting point is 202–203° (after previous softening). The compound is somewhat more soluble than the dihydro-β-derivative. It is epiphasic in the partition test. A 0.1% benzene solution did not show optical activity in a 1-dm. tube. The analyzed samples were dried in high vacuum at 56°. Of three independent preparations two were free of ash; for the third the results are corrected for 0.6% ash. *Anal.* Calcd. for $C_{40}H_{58}$: C, 89.15; H, 10.85; mol. wt., 539. Found: C, 88.92, 89.10, 89.37; H, 11.36, 11.08, 10.88; mol. wt., 487, 478, 516 (a considerable fraction underwent isomerization in the camphor melt).

Catalytic Hydrogenation.—8.228 mg. absorbed 3.80 of hydrogen (25°, 738 mm.) in the presence of 19 mg. of PtO_2; 5.095 mg. with 14.5 mg. of PtO_2 absorbed 2.275 ml. (24°, 743 mm.). $C_{40}H_{58}$. Found: 9.9 and 9.7 double bonds.

Isopropylidene Group.—On ozonolysis 11.21 and 11.94 mg. of substance gave acetone corresponding to 0.66 and 0.64 ml. of N/20 iodine. $C_{40}H_{58}$. Found: 0.26 and 0.24 $(CH_3)_2C{=}$.

For spectra see Table II for spectral curves the foregoing paper.[5]

The concentration of 5,6-dihydro-α-carotene in petroleum ether can be estimated in the Pulfrich instrument using light filter S45

k	0.2	0.4	0.6	0.8	1.0
c	0.088	0.182	0.282	0.382	0.477

On the Tswett column dihydro-α-carotene can be recognized easily by its bright greenish yellow-color of moderate intensity. Compared with it, α- or β-carotene zones appear to be intensely orange. When the petroleum ether contains 5% acetone, the dihydro-α-carotene is rapidly washed down into the chromatographic filtrate while the other carotenes still form well-defined zones. Depending upon the quality of the calcium hydroxide, the addition of 0.5% acetone may be advantageous for the separation of the two dihydro compounds from each other.

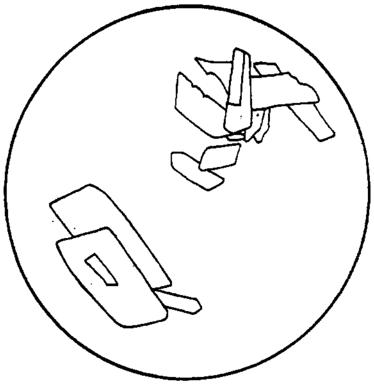

Fig. 2.—5,6-Dihydro-α-carotene, crystallized from carbon disulfide and ethanol.

cis-trans-Isomerization of 5,6-Dihydro-α-carotene

(a) **On Standing or Refluxing.**—A solution of 3 mg. in 20 ml. of petroleum ether was found practically unchanged after one to two hours of standing at 27°. The colorimetric ratio, unchanged all-*trans*:neo-forms with increased adsorbability:neo-forms with decreased adsorbability was found after chromatography as follows

Hours of standing	2	20	65
Ratio	100:0:0	92:6:2	79:17:4

At 5° only 5% of the starting material isomerized within 65 hours and most of the isomers formed were adsorbed above the all-*trans* pigment.

A solution of 3 mg. of dihydro-α-carotene in 50 ml. of petroleum ether, in an all-glass apparatus, was refluxed in a slow stream of carbon dioxide for thirty minutes. After cooling it was developed on a column (20 × 3.8 cm.) with petroleum ether

22 orange, traces, unidentified, irreversible
20 pale yellow, neo T: 460, 432.5 mμ (with iodine 466.5, 438 mμ)
10 colorless
30 greenish-yellow, neo U: 464.5, 436.5 mμ (468, 438.5 mμ)
15 slightly colored
60 bright greenish-yellow, unchanged all-*trans*: 470, 440.5 mμ (468, 438.5 mμ)
5 lighter yellow, neos A + B: 467, 438.5 mμ (466.5, 438.5 mμ)

The colorimetric ratio: Unchanged all-*trans*:neo T: neo U:neo-forms A + B was 64:3:27:6.

The lowest zone separated on rechromatography into a major portion, neo-dihydro-α-carotene A, showing maxima at 462.5, 435 mμ, and a well-defined minor zone below it, neo B, with maxima at 455.5, 425 mμ. Both gave the expected equilibrium spectra on addition of iodine.

(b) **Isomerization by Iodine Catalysis at Room Temperature.**—Ten milligrams of 5,6-dihydro-α-carotene in 50 ml. of petroleum ether was mixed with a solution of 0.2 mg. of iodine, and chromatographed 30 min. later on a column (27 × 5.8 cm.)

40 colorless
20 yellow, neo T: 461.5, 432.5 mμ (with iodine 467.5, 438.5 mμ)
10 colorless
32 yellow, neo U: 465.5, 437.5 mμ (467.5, 440 mμ)
10 colorless
45 yellow, unchanged all-*trans*: 469.5, 441 mμ (468, 439 mμ)
3 slightly colored
20 yellow, neo A: 463, 436 mμ (467, 437.5 mμ)
8 yellow, neo B: 459.5, 429 mμ (465, 436 mμ)

Each zone was cut out, eluted, treated with iodine and chromatographed thirty minutes later. The relative photometric values of the respective stereoisomers formed are listed in Table IV.

TABLE IV

RELATIVE PHOTOMETRIC VALUES OF 5,6-DIHYDRO-α-CAROTENE AND OF ITS STEREOISOMERS AS FORMED BY IODINE CATALYSIS OF THE INDIVIDUAL ISOMERS

<table>
<tr><th rowspan="2">Starting material</th><th colspan="5">Relative photometric values (%)</th></tr>
<tr><th>neo T</th><th>neo U</th><th>all-<i>trans</i></th><th>neo A</th><th>neo B</th></tr>
<tr><td>Neo T</td><td>28</td><td>15</td><td>55</td><td colspan="2">8</td></tr>
<tr><td>Neo U</td><td>5</td><td>22</td><td>55</td><td colspan="2">18</td></tr>
<tr><td>All-<i>trans</i></td><td>4</td><td>20</td><td>56</td><td>16</td><td>4</td></tr>
<tr><td>Neo A</td><td>2</td><td>17</td><td>49</td><td>26</td><td>6</td></tr>
<tr><td>Neo B</td><td>1</td><td>19</td><td>64</td><td>11</td><td>5</td></tr>
</table>

(c) **Isomerization with Hydriodic Acid and Hydrochloric Acid.**—A solution of 5 mg. of 5,6-dihydro-α-carotene was shaken with 10 ml. of diluted hydriodic acid (1 volume of concd. acid and 4 volumes of water) for thirty minutes. The chromatogram (26 × 5.8 cm.) was very similar to that obtained with iodine, but the minor isomer T was missing. The colorimetric ratio, unchanged all-*trans*: neo U: neo-forms A + B was 83:16:1. Another experiment gave with undiluted acid the chromatogram

65 faintly colored, irreversible
20 pale greenish-yellow (neo T and irreversible products)
15 colorless
30 greenish-yellow, neo U: 463, 435.5 mμ (466, 437 mμ)
15 colorless
40 greenish-yellow, unchanged all-*trans*: 467.5, 437 mμ (466, 436.5 mμ)
20 lighter yellow, neo forms A + B: 462, 434.5 mμ (466 436.5 mμ)

The colorimetric ratio, unchanged all-*trans*:neo U:neo-forms A + B was 66:24:10. If concd. hydrochloric acid was applied, the ratio, unchanged all-*trans*: neo T:neo U: neo-forms A + B was 58:3:21:18.

(d) **Isomerization by Melting.**—Three milligrams of 5,6-dihydro-α-carotene was melted in a sealed tube filled with carbon dioxide and kept in a bath at 210° for fifteen minutes. After cooling in ice-water the petroleum ether solution was chromatographed (column, 20 × 3.8 cm.). About three-fourths of the original colorimetric value disappeared during the experiment. The presence of colorless cleavage products was indicated by a greenish fluorescence of the chromatographic filtrate in ultraviolet light.

35 colorless
17 pale yellow, neo T: 459, 432.5 mμ (with iodine 465, 437.5 mμ)
10 colorless
30 yellow, neo U: 465.5, 437.5 mμ (467.5, 437.5 mμ)
7 colorless
20 pale yellow, neo V: 458, 431 mμ (465.5, 437.5 mμ)
5 almost colorless
30 yellow, unchanged all-*trans*: 468.5, 439.5 mμ (467, 437.5 mμ)
5 yellow, neo-forms A + B: 459, 430 mμ (467.5, 437 mμ)

The colorimetric ratio was: all-*trans*: neo T:neo U:neo V:neo-forms A + B = 45:8:26:17:4.

Summary

If petroleum ether solutions of α- or β-carotene are shaken with cold, commercial concd. hydriodic acid, a subsequent chromatogram shows no unchanged starting material but smaller amounts of some new pigments, two of which have been crystallized. These stable compounds were characterized as 5,6-dihydro-β-carotene and 5,6-dihydro-α-carotene on the basis of analyses, catalytic hydrogenation, isopropylidene estimation, and spectroscopic measurements in the region extending from 220 to 500 mμ. The dihydro compounds show the usual phenomena of reversible *trans–cis*-isomerization, on heating, melting, acid or iodine catalysis; furthermore the spectroscopic "*cis*-peak" effect described in the foregoing paper[5] was observed.

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