Anal. Calcd. for $C_{24}H_{22}NOBr$: C, 68.57; H, 5.28. Found: C, 68.80, 68.52; H, 5.36, 5.42.

This bromo amino ketone reacted with sodium ethoxide in the usual way to give a red oily product which was probably the α-tetrahydroisoquinolinobenzalacetophenone. All attempts to crystallize this oil failed.

When a sample of (IV) was allowed to stand in absolute alcohol at room temperature for one week a small amount (about 20%) of the addition product decomposed[2f] to give α-bromobenzalacetophenone and tetrahydroisoquinoline. This was shown by the isolation of a 10% yield of α,β-ditetrahydroisoquinolinobenzylacetophenone (V), m. p. 184–186°, identical with that prepared from α,β-dibromobenzylacetophenone as described below.

α,β-Ditetrahydroisoquinolinobenzylacetophenone (V). —In 20 ml. of absolute alcohol was suspended 5 g. (0.0136 mole) of α,β-dibromobenzylacetophenone and the mixture cooled to 0°. Tetrahydroisoquinoline (8 g. (0.060 mole)) was added rapidly while shaking. The solution at first remained white, but then changed to an orange color. After allowing it to stand for two days at room temperature, during which time the mixture was shaken and worked with a spatula, the white precipitate was filtered off and washed well with petroleum ether and then with water. The crude product was recrystallized twice from benzene–petroleum ether (b. p. 80°) mixtures to give 4.4 g. of a white product.

α-Tetrahydroisoquinolino-β-morpholinobenzylacetophenone (VI).—In 15 ml. of dry ether and 5 ml. of absolute alcohol, were suspended 5 g. (0.012 mole) of (IV) and 3.6 g. (0.024 mole) of morpholine added. After standing overnight at room temperature the red-colored solution was filtered, the precipitate washed with petroleum ether and then with water. After three recrystallizations from a chloroform–alcohol mixture and two from a benzene–petroleum ether mixture, 1.7 g. of pale yellow crystals was obtained.

Hydrolysis.—Acid hydrolysis (40 ml., 15% sulfuric acid) of the diamino ketone (VI) (4.0 g.) in the ordinary manner[2e] gave 1.65 g. of ω-tetrahydroisoquinolinoacetophenone, identical with a sample prepared from ω-bromoacetophenone.[3]

Other mixed diamino ketones prepared by essentially[6] this same procedure were: (VII) from (IV) and two equivalents of tetrahydroquinoline; (VIII) from α-bromo-β-morpholinobenzylacetophenone[2b] and tetrahydroisoquinoline (mixed m. p. of (VI) with (VIII), 150–163°; hydrolysis of (VIII) gave mainly ω-morpholinoacetophenone[2b] and traces of ω-tetrahydroisoquinolinoacetophenone[3] along with tetrahydroisoquinoline and benzaldehyde); (IX) from α-bromo-β-piperidinobenzylacetophenone[4] and tetrahydroisoquinoline (hydrolysis of (IX) gave ω-piperidinoacetophenone, isolated as its hydrochloride[2d]); (X) from α-bromo-β-piperidinobenzylacetophenone[4] and cyclohexylamine.

Summary

1. The preparation of one new α-bromo-β-amino ketone and nine new diamino ketones has been described.

(6) The amount of absolute alcohol in the reaction medium and the time for reaction varied slightly in each individual case.

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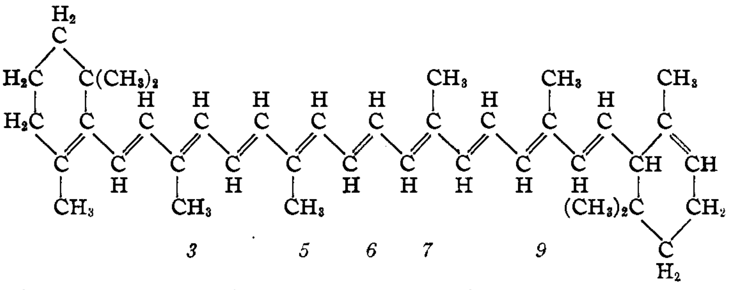
cis–trans Isomerization and *cis*-Peak Effect in the α-Carotene Set and in Some Other Stereoisomeric Sets

BY L. ZECHMEISTER AND A. POLGÁR

As far as we know, no up-to-date study of the stereoisomerization of α-carotene[1] is available while a more detailed investigation has been presented for β-carotene.[2] The α-carotene molecule includes five double bonds which are available for *trans*→*cis* rotations (see the formula), the number of stereoisomers being thirty-two.[3]

It was first assumed[4] that the all-*trans* member of a stereoisomeric hydrocarbon set possesses greater adsorption affinity than any other member of the set but this postulate must now be abandoned for both carotene sets mentioned.

A re-investigation of α-carotene showed the presence of the eleven isomers listed in Table I of which neo-α-carotene U and W have been crystallized.



All-*trans*-α-carotene. (The double bonds available for a *trans*→*cis* shift are numbered.)[5]

The ratio of stereoisomers is dependent on the method of isomerization (Table II).

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(1) A. E. Gillam, M. S. El Ridi and S. K. Kon, *Biochem. J.*, **31**, 1605 (1937).

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

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

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

(5) The following *nomenclature* is proposed. Each double bond of the *conjugated system* will be assigned an *italicized* number in order to avoid confusion with the numbering of carbon atoms: *e. g.*, *3,6*-di-*cis*-β-carotene. The lowest number will be given to the double bond in the β-ionone ring or, if the double bond of this ring is not part of the chromophore, to the conjugated double bond nearest the β-ionone ring. In the absence of such a system an α-ionone ring receives preference over an aliphatic terminal group.

TABLE I

VISUALLY DETERMINED SPECTRAL MAXIMA OF OBSERVED MEMBERS OF THE STEREOISOMERIC α-CAROTENE SET IN THE SEQUENCE OF DECREASING ADSORPTION AFFINITY

Name	Maxima in petroleum ether (b. p. 60–70°), mμ	
Neo-α-carotene U	471.5	441.5
Neo-α-carotene V	465.5	437
Neo-α-carotene W	470.5	441
Neo-α-carotene X	463.5	435
Neo-α-carotene Y	467.5	437
All-*trans*-α-carotene	477	446.5
Neo-α-carotene A	468.5	439
Neo-α-carotene B	466.5	437
Neo-α-carotene C	472.5	442.5
Neo-α-carotene D	460	432
Neo-α-carotene E	461.5	433.5

TABLE II

RELATIVE COLORIMETRIC VALUES OF THE MAIN PRODUCTS OF STEROISOMERIZATION OF ALL-*trans*-α-CAROTENE BY VARIOUS METHODS

Method	All-*trans*	Neo U	Neo W	Neo B	Other isomers
Refluxing	92	4	—	4	—
Iodine catalysis (in light)	65	8.5	8	13	5.5
Acid catalysis (10% HI)	64.5	5	24	4.5	2
Acid catalysis (37% HCl)	50	13	15	9	13
Melting crystals	35	12	19	14	20

For effective iodine catalysis diffuse daylight or illumination is required.

Exposure to sunshine ("insolation") in the absence of catalysts is a recent addition to isomerization methods. Even when gradual destruction takes place, this irreversible change does not hinder the chromatographic study of the stereoisomerization. We wish to emphasize that the configuration of no all-*trans* or partially *cis* carotenoid tested so far is photo-resistant. The rate of the steric changes is dependent on the initial configuration. Natural β-carotene is somewhat more photo-stable than α-carotene.

Upon a forty-five minute insolation of petroleum ether solutions the following percentages of the recovered pigment refer to sterically unchanged starting material: all-*trans*-α-carotene, 93%; neo V, 43%; neo W, 33%; and neo B, 8%; all-*trans*-β-carotene, 98%; neo U, 37%; and neo B, 5%.

A study of the "*cis*-peak" effect has been of aid in establishing the most probable configuration of some stereoisomers. While it had been found by several authors that the extinction maxima of the ordinary carotenoids in the visible region decrease upon isomerization,[6] we observed that a new marked maximum develops simultaneously at some wave length between 320 and 380 mμ ("*cis*-peak").[7] The contribution of individual stereoisomers to the height of the *cis*-peak observed in equilibrium mixtures is very unequal in the β-carotene set. The greatest contribution is made by those neo-compounds which are adsorbed below the all-*trans* pigment while the U isomer contributes much less. We have now carried out a more detailed differentiation of individual *cis*-peak effects in the β-carotene, α-carotene, lutein, and lycopene sets as shown in Figs. 1 to 4.

If an all-*trans* carotenoid undergoes spatial changes, the bending of the molecule causes two different, easily observable optical effects which may be helpful in the assignment of a definite configuration to each isomer formed: (a) the visible maxima shift toward shorter wave lengths and (b) a *cis*-peak with characteristic height appears in the ultraviolet region. In possession of a sound theoretical basis, effect (a) would give us the number of *cis* double bonds and (b) would determine the general shape of the molecule, *i. e.*, the position of the *cis* bonds in the conjugated system. The main difficulty at present lies in the lack of precise information concerning the possible dependence of (a) on the position of the double bonds which have completed a *trans*→*cis* rotation. On the basis of the experimental material available it seems that the *trans*→*cis* rotation of one double bond displaces the longest wave length maximum in petroleum ether about 5 mμ toward shorter wave lengths. If this spectral shift equals approximately 10 mμ the presence of two *cis* double bonds is probable, etc.

It has been reported by Pauling, LeRosen, Schroeder and the authors[8] that a stereoisomer showing a very high *cis*-peak must possess a *cis* double bond at (or near) the center of the conjugated system. It was pointed out that as a rough approximation the intensity of the *cis*-peak can be taken proportional to the square of the distance between the center of the chromophore and the mid-point of the straight line between its two ends. The models show that the rotation of a second double bond into *cis* position will straighten the molecule considerably and accordingly will diminish the *cis*-peak. This effect is essentially independent of the position of the second double bond. The conditions are more complicated in the case of a chromophore containing an even number of conjugated double bonds (α-carotene) than in that of, for example, β-carotene. The center of the α-carotene chromophore is a single bond; two "central" double bonds, *5* and *6*, are present, each of which has four other conjugated double bonds on one side and five on the other. These "central" double bonds, while structurally different in α-carotene, are equivalent from the

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[6] *Cf. e. g.*, some curves of original and isomerized pigments in the visible region: H. H. Strain, "Leaf Xanthophylls," Carnegie Inst. of Washington (1938); *J. Biol. Chem.*, **127**, 191 (1939); B. W. Beadle and F. P. Zscheile, *ibid.*, **144**, 21 (1942); 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).

[7] L. Zechmeister and A. Polgár, THIS JOURNAL, **65**, 1522 (1943).

[8] L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgár and L. Pauling, *ibid.*, **65**, 1940 (1943).

stereochemical viewpoint. Therefore, two of the mono-*cis*-α-carotenes can be expected to have much higher *cis*-peaks than any other isomer. Models show that the molecule of *5,6*-di-*cis*-α-carotene, the isomer which contains both "central" double bonds in *cis* position, is considerably straighter than either *5*- or *6*-mono-*cis*-α-carotene.

The following discussion in which some stereoisomers are tentatively given spatial structures is based on the foregoing considerations and on the optical data summarized in Table III. (Should stereoisomers be detected in the future which possess higher *cis*-peaks than any of the members of the set known at the present, a revision of our assignments of spatial structures in that particular set would become necessary.)

TABLE III

TYPICAL SPECTROSCOPIC DATA FOR SOME MEMBERS OF THE STEREOISOMERIC β-CAROTENE, α-CAROTENE, LUTEIN, AND LYCOPENE SETS

Stereo-isomeric set	Member of the set	Difference of the visually established longest wave length maximum from that of the all-*trans* form, in petroleum ether (mμ)	Molecular extinction coefficient, in hexane, at the respective *cis*-peaks, $E^{\text{mol.}}_{1\text{cm.}} \times 10^{-4}$	Difference of $E^{\text{mol.}}_{1\text{cm.}}$ for member of the set and the all-*trans* form
β-Carotene	neo U	5	1.3	0.5
	neo V	13.5	0.8	0
	all-*trans*	0	0.8	0
	neo B	10.5	3.4	2.6
	neo E	8.5	3.4	2.6
α-Carotene	neo U	5.5	1.2	0.4
	neo V	11.5	1.1	0.3
	neo W	6.5	1.6	0.8
	neo X	13.5	2.7	1.9
	all-*trans*	0	0.8	0
	neo A	8.5	3.8	3.0
	neo B	10.5	3.8	3.0
	neo C	4.5	4.5	3.7
Lutein	neo A	6	4.9	4.1
	neo B	7	2.1	1.3
	all-*trans*	0	0.8	0
Lycopene	all-*trans*	0	1.4	0
	neo A	5	6.8	5.4
	neo B	8	3.7	2.3
	unnamed cryst. isomer	28	1.3	−0.1
	prolycopene	34	1.6	0.2
	all-*cis*	38.5	2.2	0.8

β-Carotene Set (Fig. 1).—The visual maxima of neo-β-carotene U differ by 5 mμ and those of neo-β-carotene V by 13.5 mμ from the positions of the maxima of all-*trans*-β-carotene and indicate thereby that neo U is a mono-*cis* and neo V a di-*cis* compound. Neither of them can contain a *6*-*cis* double bond because of their low *cis*-peaks and relatively high thermostability. We suggest as the most probable configurations:[9] *3*-mono-*cis*-β-carotene for neo U, and *3,9*-di-*cis*-β-carotene for neo V; however, for neo V the configuration *5,7*-di-*cis* cannot be excluded.

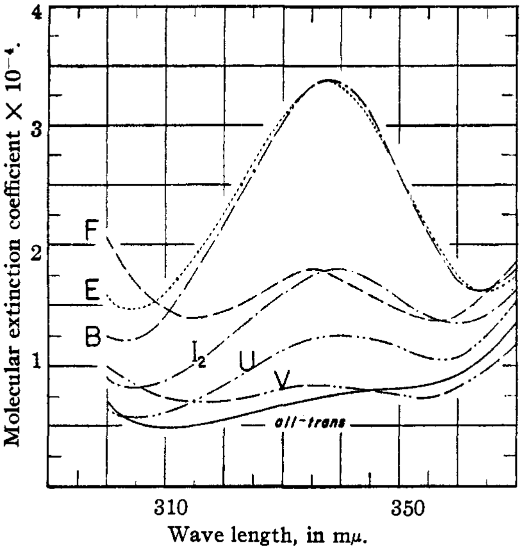

Fig. 1.—Molecular extinction curves of some β-carotene stereoisomers in the *cis*-peak region (in hexane). I_2 indicates the equilibrium mixture obtained upon iodine catalysis.

Neo-β-carotene B, a main isomer, is interpreted as a di-*cis* compound which has one of its *cis* bonds in the *6*-position; this is substantiated by the high *cis*-peak and the relatively slight thermostability. The most probable configuration for neo B is *3,6*-di-*cis*-β-carotene because *5,6* must be definitely eliminated after a consideration of the *cis*-peak and models. The minor isomer, neo-β-carotene E, belongs to the same type as neo B but because of its lability we do not wish to make suggestions concerning its configuration.

α-Carotene Set (Fig. 2).—The neo-α-carotenes U, V, and W show considerable thermostability and very moderate *cis*-peaks. The isomers U and W seem to contain one *cis* double bond while V must reasonably be interpreted as a di-*cis* compound. The most probable configurations are: neo U = *9*-mono-*cis*-α-carotene, neo W = *3*-mono-*cis*-α-carotene, and neo V = *3,9*-di-*cis*-α-carotene. Neo-α-carotene X, a di-*cis* compound, was preponderantly formed upon mild heating of a neo U solution. Therefore, we suggest that one of its *cis* double bonds, *viz.*, *9*, is identical with that in neo U. Since, as will be shown, the configurations *5,9* and *6,9* must be allocated to other isomers, neo-α-carotene X possessing a medium *cis*-peak is probably *7,9*-di-*cis*-α-carotene. Neo-α-carotene C, a labile minor isomer, seems to be *6*-mono-*cis*- or *5*-mono-*cis*-α-carotene.

One of the main observed isomers, neo B, has

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[9] Before the *cis*-peak was found we stated that neo-β-carotene U possibly contains a *6*-*cis* double bond (footnote 2). This assumption must be abandoned.

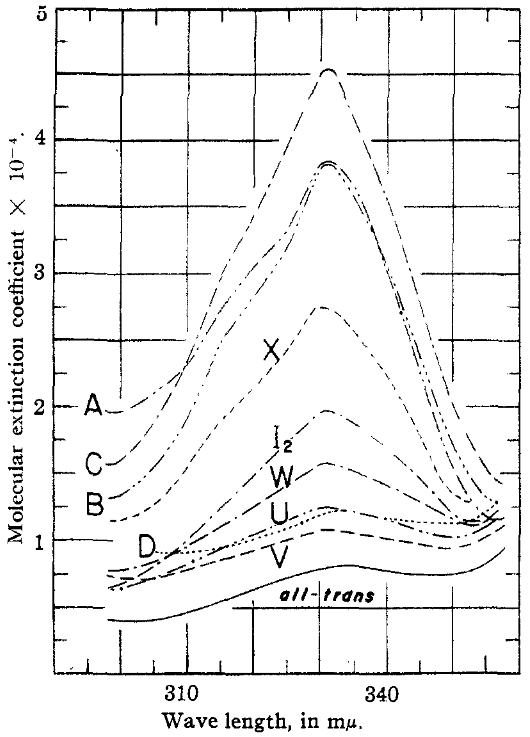

Fig. 2.—Molecular extinction curves of some α-carotene stereoisomers in the *cis*-peak region (in hexane). I_2 indicates the equilibrium mixture obtained upon iodine catalysis.

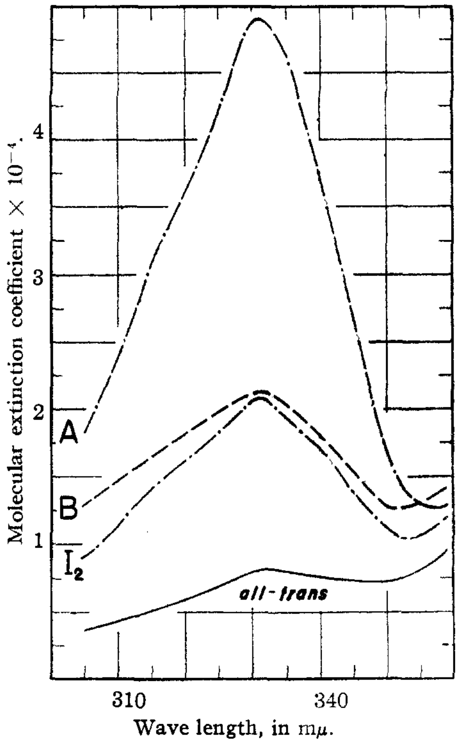

Fig. 3.—Molecular extinction curves of some lutein stereoisomers in the *cis*-peak region (in hexane). I_2 indicates the equilibrium mixture obtained upon iodine catalysis.

approximately the same *cis*-peak and in the visible region, the same spectral difference from the all-*trans* maxima as neo-β-carotene B. The great height of its *cis*-peak strongly suggests that one of its *cis* positions is central, the other peripheral, since neither two peripheral nor two central *cis* double bonds would explain the height of the peak observed. The very fact, however, that in α-carotene we find two stereochemically equivalent but structurally different central double bonds, leaves for neo-α-carotene B two spatial possibilities open which are, according to the models, *5,9-* and *6,9*-di-*cis*-α-carotene. Since the neo-α-carotenes A and B have practically identical *cis*-peaks, it is reasonable to say that for these two isomers the following four configurations are possible: *5,9*, *6,9*, *3,5*, and *3,6*.

Lutein Set (Fig. 3).—Only the two main isomers, neo A and B, have been investigated. On the basis of Table III neo-lutein A is very probably *6-* or *5*-mono-*cis*-lutein. The extinction at the *cis*-peak of neo-lutein B is less than half that of neo A. Neo B is probably *3-* or *9*-mono-*cis*-lutein.

Lycopene Set (Fig. 4).—As previously mentioned,[8] neo-lycopene A, which possesses the highest *cis*-peak so far observed, must be interpreted as *6*-mono-*cis*-lycopene. The isomer B is evidently a di-*cis* compound which could well be *1,6-* or *3,6*-di-*cis*-lycopene.

It will be an important task for the future to establish the precise nature of the dependence of adsorption affinity on the configuration of polyenes. At present only the following remarks can be made with reference to the adsorbability of stereoisomeric α- and β-carotenes (on calcium hydroxide). If the tentatively proposed spatial structures are correct, it follows that, when the position of the *cis* double bonds is *3* or *9*, the mono-*cis* compounds observed possess greater adsorption affinity than the all-*trans* form. In contrast, mono-*cis* isomers with one centrally located *cis* double bond show a decreased adsorbability. The chromatographic behavior of *3,9*-di-*cis* compounds is like that of the *3-* or *9*-mono-*cis* isomers. No simple rule is available for the case in which one peripheral and one central double bond are present. Isomers with two centrally located *cis* double bonds or with three *cis* double bonds in any combination observed so far possess definitely weaker adsorption affinity than the all-*trans* member of the α- and β-carotene sets.

Experimental

Methods.—The pigment solutions were chromatographed on calcium hydroxide[10] (Shell brand lime, chemical

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[10] Since calcium hydroxides from various sources show a great difference in the rate of solvent flow, the following test, suggested by Dr. A. L. LeRosen, is used in our laboratory for standardization: a 7 × 2 cm. column is prepared, petroleum ether is sucked into it, and the time needed for the solvent to travel 50 mm. down the column is measured. A result of 35 to 70 seconds is adequate (pressure 25 mm. Hg).

hydrate; 98% through 325 mesh). For development petroleum ether (Skellysolve B, b. p. 60–70°) containing 0.5% acetone was used. The figures on the left side of the chromatograms described below denote width of the zones in mm. Petroleum ether–alcohol mixtures are suitable eluents. The visual spectral maxima were determined with an Evaluating Grating Spectroscope (Zeiss, light filter BG-7, 2 mm. thick) in petroleum ether unless otherwise indicated. For colorimetric estimations a Pulfrich Gradation Photometer was used (petroleum ether, filter S47). The extinction coefficients were determined in a Beckman Photoelectric Spectrophotometer.[11] For these experiments "Hexane" (Eastman Kodak Co. from petroleum, "practical") was purified by shaking with fuming sulfuric acid and then with alkaline permanganate, and by fractionating; b. p. 62–65°.

The treatment of isomers which were available as solutions was described earlier.[7,8] The curves in Figs. 1 to 4 may be in error to the extent of ±1 to 5% at the maxima.

All pigments listed in the chromatograms as members of the α-carotene set shifted their visually observed spectral maxima to 473.5, 443.5 mμ (±0.5 mμ) after addition of iodine.

(a) ***cis–trans* Isomerization of α-Carotene Solutions on Standing or Refluxing.**—A solution of 3 mg. of pure α-carotene in 30 ml. of petroleum ether was found chromatographically to be nearly unchanged after remaining two hours in diffuse light. Even upon longer standing the extent of isomerization is very moderate. The neo-compounds formed were adsorbed partially above and partially below the unchanged portion. In petroleum ether solution the colorimetric ratio, unchanged all-*trans*: neo-forms was 98:2 after a day. At 5° and in the dark 1% of the α-carotene isomerized within three days.

A 50-ml. petroleum ether solution containing 5 mg. of α-carotene was refluxed in carbon dioxide for thirty minutes in an all-glass apparatus. The cooled solution was developed on a 24 × 5.8 cm. column.

35	colorless
45	pale orange, neo U: 470.5, 440.5 mμ
17	colorless
45	orange, unchanged all-*trans:* 476, 445 mμ
2	colorless
20	pale orange, neo B . . . :[12] 469.5, 440.5 mμ

The colorimetric ratio was, unchanged all-*trans* : neo U:neo B . . . = 92:4:4.

(b) ***cis–trans* Isomerization of α-Carotene by Iodine Catalysis at Room Temperature.**—To the solution of 6 mg. of homogeneous α-carotene in 50 ml. of petroleum ether a solution of 0.1 mg. of iodine was added and the mixture chromatographed thirty minutes later (24 × 4.8 cm.).

10	colorless
40	pale orange, neo U: 471, 442 mμ
7	colorless
10	light yellow, neo V: 466, 437 mμ
7	colorless
40	yellow, neo W: 470, 440.5 mμ
2	colorless
36	light orange, unchanged all-*trans:* 476, 447 mμ
5	colorless
25	yellow, neo B: 467.5, 438 mμ
2	colorless
2	pale yellow, neo C: 472.5, 442.5 mμ
2	colorless
5	pale yellow, neo D: 460, 432 mμ
2	colorless
5	pale yellow, neo E: 461.5, 433.5 mμ

The colorimetric ratio was, unchanged all-*trans*:neo U:neo V:neo W:neo B:neo C:neo D:neo E = 51.5:14.5:3:15.5:13:0.5:1:1.

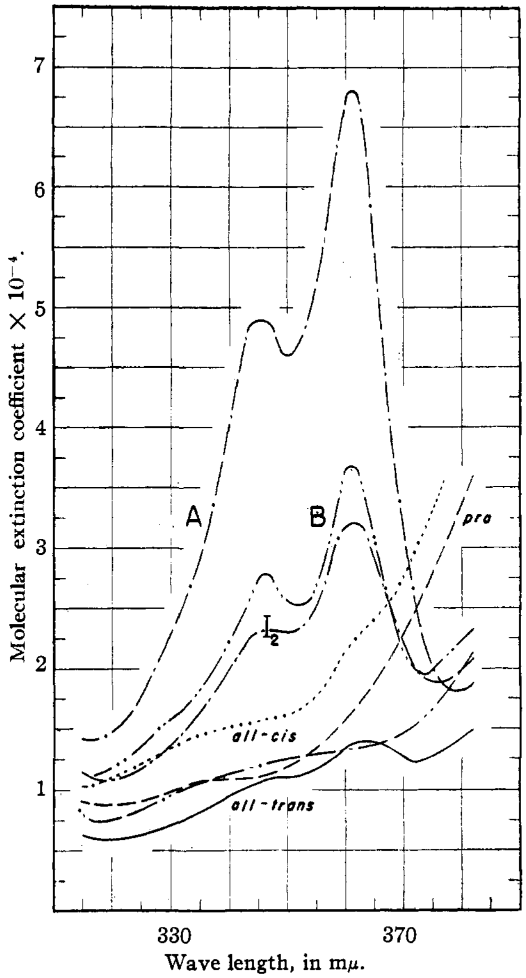

Fig. 4.—Molecular extinction curves of some lycopene stereoisomers in the *cis*-peak region (in hexane). I₂ indicates the equilibrium mixture obtained upon iodine catalysis, and . . – – . . an unnamed crystallizable stereoisomer (first absorption band at 476 mμ).

Similar isomerization experiments were carried out with the respective neo pigments (Table IV).

TABLE IV

RELATIVE COLORIMETRIC VALUES OF α-CAROTENE AND OF SOME OF ITS STEREOISOMERS FORMED BY IODINE CATALYSIS AT 25°

Starting material	Relative colorimetric values (% of the recovered pigment) neo U	neo V	neo W	all-*trans*	neo B	neo C . . .
Neo U	11	3.5	21	49.5	12	3
Neo V	11.5	4	19.5	50	13	2
Neo W	15.5	3.5	18.5	43.5	17	2
All-*trans*	14.5	3	15.5	51.5	13	2.5
Neo B	10.5	3	15	57	12.5	2
Neo C + D + E	10	2.5	21	51.5	9.5	5.5

(c) ***cis–trans* Isomerization of α-Carotene with Hydriodic Acid and Hydrochloric Acid.**—A homogeneous solution containing 7.5 mg. of pigment in 30 ml. of petroleum ether was shaken for thirty minutes with 15 ml. of hydriodic acid (Merck Reagent, 55–58% HI, diluted 1:1) in a carbon dioxide atmosphere. The pigment solution

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[11] H. H. Cary and A. O. Beckman, *J. Optical Soc. Am.*, **31**, 683 (1942).

[12] The dots . . . indicate that the zone of the neo-compound designated by the letter included minor isomers, whose separation was unsatisfactory. In this case the neo-forms C, D, and perhaps E, were present.

was washed acid-free. The chromatogram was similar to that obtained upon iodine catalysis (section b). Above neo-α-carotene U there appeared some irreversibly formed pigment (<1%) but no 5,6-dihydro-α-, or 5,6-dihydro-β-carotene[13] was present. The all-*trans*-α-carotene recovered from a larger scale experiment showed its original rotatory power.

The colorimetric ratio was, unchanged all-*trans*:neo U: neo V:neo W:neo B:neo C... = 64.5:5:1.5:24:4.5:0.5.

In a parallel experiment commercial concentrated hydrochloric acid was applied and the following chromatogram obtained (24 × 4.8 cm.).

35	several yellow, irreversible pigments
25	pale orange, neo U: 472, 442 mμ
2	colorless
3	light yellow, neo V: 465.5, 437 mμ
25	colorless
30	yellow, neo W: 470.5, 441.5 mμ
3	colorless
25	orange, unchanged all-*trans*: 477.5, 447 mμ
5	colorless
12	light orange, neo A: 468.5, 439.5 mμ
2	almost colorless
10	yellow, neo B: 467, 438 mμ
2	almost colorless
4 5	pale orange, neo C: pale yellow, neo D: } 467, 437.5 mμ

The colorimetric ratio was, unchanged all-*trans*:neo U: neo V:neo W:neo A:neo B:neo C... = 49:13:2:15:8: 9:4.

The quantity of the *irreversibly formed pigments* amounted to about 3% of the total. Longer shaking with the acid promotes their formation (79% after 3 hours). Chromatography on calcium carbonate and subsequent crystallization from benzene-methanol yielded a few milligrams of long orange needles, m. p. 170° (cor.). In the partition test this pigment behaved like cryptoxanthin. The visual maxima follow: in carbon disulfide, 507, 473 mμ (with iodine, 504, 471.5 mμ); in benzene, 488, 456.5 mμ (486, 454.5 mμ); in petroleum ether (b. p. 60–70°), 477, 446.5 mμ (474, 443.5 mμ); and in ethanol, 477.5, 448 mμ (477.5, 448 mμ).

(d) ***cis-trans* Isomerization of α-Carotene by Melting.**—Three milligrams of pigment was melted in a sealed tube filled with carbon dioxide and kept in a bath at 195–200° for fifteen minutes. After cooling in ice water the petroleum ether solution was chromatographed (23 × 4.8 cm.).

20	colorless
12	pale orange, neo U: 472.5, 442.5 mμ
22	colorless
5	yellow, neo V: 466.5, 437.5 mμ
6	colorless
10	pale orange, neo W: 470.5, 440.5 mμ
2	colorless
3	yellow, neo X: 463.5, 434 mμ
5	colorless
17	light orange, unchanged all-*trans*: 476.5, 447 mμ
5	colorless
10	yellow, neo B: 466, 437 mμ
5	almost colorless
15	pale yellow, neo D...: 462, 433.5 mμ

The colorimetric ratio was, unchanged all-*trans*:neo U:neo V: neo W:neo X:neo B:neo D... = 35:12:6:19:7:14:7.

(e) **Neo-α-carotene U.**—To a solution of 110 mg. of α-carotene in 500 ml. of petroleum ether 3 mg. of iodine was added and, after standing at 25° for an hour, the solution chromatographed on two 29 × 8 cm. columns. The light orange neo U zone adsorbed near the top was cut out separately while the other pigments were combined, treated with iodine again and chromatographed. The neo U zones of both chromatograms were eluted together. This pigment was rechromatographed twice (29 × 8 cm.), transferred into petroleum ether and evaporated. The oily residue was dissolved in the minimum amount of benzene, mixed in a centrifuge tube with some methanol and dipped into a dry ice–acetone mixture for a few seconds. Upon scratching, crystals appeared (yield, 26.2 mg. = 24%). A single treatment of α-carotene with iodine yields about 14%. Neo-α-carotene U forms an orange crystal powder; under the microscope light orange 0.01 mm. long prisms appeared (Fig. 6); m. p. 65° (cor.). The compound is more soluble than natural α-carotene. The visually determined spectra are included in Table V. Upon addition of iodine the same maxima were observed as in case of a catalyzed all-*trans*-α-carotene solution, *viz.*, in carbon disulfide, 505.5, 474 mμ; in benzene, 488, 455.5 mμ; in chloroform, 486.5, 454 mμ; in petroleum ether, 474.5, 444 mμ; and

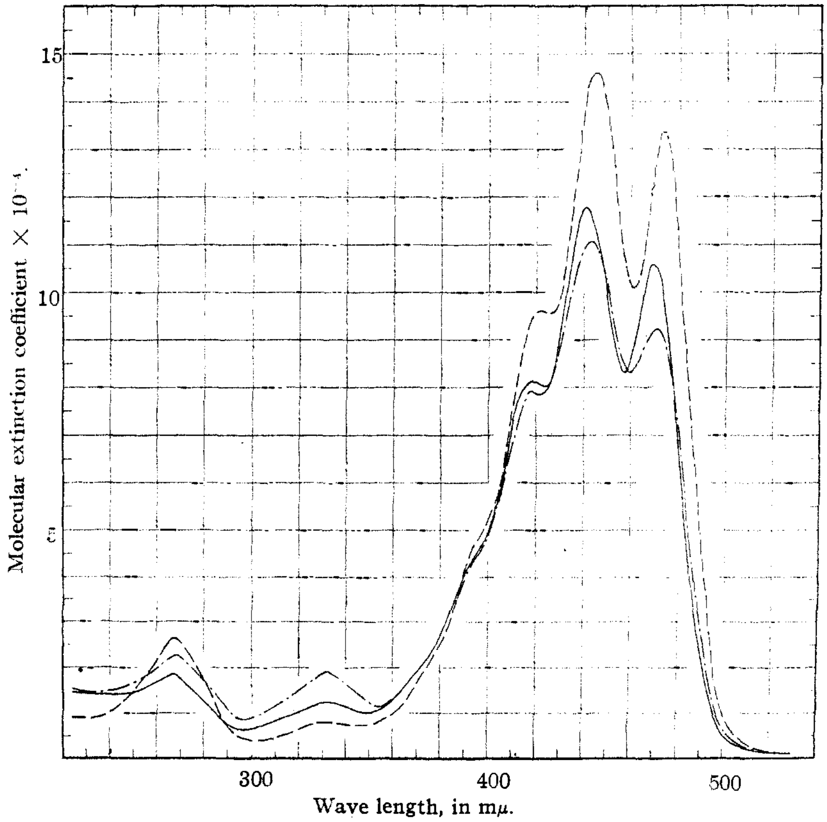

Fig. 5.—Molecular extinction curve of neo-α-carotene U, compared with that of α-carotene, in hexane: ——, neo-α-carotene U; –·–·–, neo-α-carotene U after iodine catalysis; - - - -, all-*trans*α-carotene.

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[13] A. Polgár and L. Zechmeister. This Journal, 65, 1528 (1943).

TABLE V

VISUALLY DETERMINED SPECTRAL MAXIMA OF NEO-α-CAROTENE U AND NEO-α-CAROTENE W COMPARED WITH THOSE OF ALL-*trans*-α-CAROTENE

Solvent		Neo-α-carotene U, mμ		Neo-α-carotene W, mμ		All-*trans*-α-carotene, mμ	
Carbon disulfide		503	470.5	502	469.5	509	475.5
Benzene		485.5	453.5	484	453.5	489.5	458
Chloroform		485	453	484	453	489.5	457
Petroleum ether	(b. p. 86–100°)	473.5	444	473	443.5	478.5	448
	(b. p. 60–70°)	471.5	441.5	470.5	441	477	446.5
Hexane		470.5	441	470	441	476	445.5
Ethanol		472.5	442	472.5	442	478.5	448.5 (blurred)

in hexane, 473.5, 443 mμ.

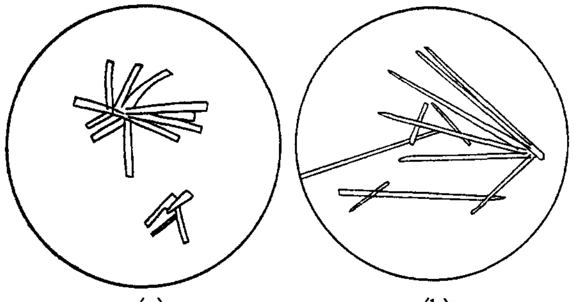
(a) (b)

Fig. 6.—Crystals of (a) neo-α-carotene U, compared with those of (b) neo-β-carotene U (from benzene and methanol).

The molecular extinction values are listed in Table VI. The deviation of readings at the main maxima of the fresh all-*trans* solution was less than 0.1%; only single series of readings were taken in other cases.

The conversion of all-*trans*-α-carotene into its U isomer decreases the specific rotation.

$$[\alpha]^{25}_{\mathrm{Cd}} = +(100 \times 0.12°):(1 \times 0.0542) = +221° \ (\pm 5\%) \quad \text{[in hexane]}$$

TABLE VI

MOLECULAR EXTINCTION COEFFICIENTS OF NEO-α-CAROTENE U AND OF ITS STEREOISOMERIC EQUILIBRIA AT THE MAXIMA (*italicized*) AND MINIMA IN HEXANE

Fresh solution		After heat isomerization		After iodine isomerization	
mμ	$E^{\mathrm{mol.}}_{1\,\mathrm{cm.}}$	mμ	$E^{\mathrm{mol.}}_{1\,\mathrm{cm.}}$	mμ	$E^{\mathrm{mol.}}_{1\,\mathrm{cm.}}$
469–70	10.6	*469*	10.3	*471*	9.2
457	8.3	457	8.3	459–60	8.4
441	11.8	*440*	11.9	*443*	11.0
424	8.1	422	7.9	422	7.9
419	8.2	*418*	8.1	*418*	7.9
350	1.03	350	1.03	354	1.17
331	1.25	*330*	1.37	*332*	1.92
298–300	0.6(4)	294	0.6(5)	298	0.8(6)
267	1.85	*268*	1.94	*269*	2.29

A thirty-minute refluxing of neo-α-carotene U in petroleum ether formed the following mixture, expressed as percentage of the recovered pigment: 70% unchanged neo U, 17% neo **X**, 6.5% neo V, 1% neo B..., and only 5.5% all-*trans*-α-carotene.

Two milligrams of neo U crystals was melted in a sealed tube filled with carbon dioxide and kept in a bath at 74° for fifteen minutes. The chromatogram of the petroleum ether solution contained 66% unchanged neo U, 23.5% all-*trans*-α-carotene (with a trace of neo A), 7% neo X, and 3.5% neo B...

(f) **Neo-α-carotene W.**—Neo W solutions collected from several experiments were twice rechromatographed; the interzone below the U layer was in no case colorless. The separation of neo W from the all-*trans* form succeeds only upon a prolonged development with petroleum ether containing 0.5% acetone. Subsequent crystallization from benzene–methanol in a dry ice–acetone bath yielded narrow prisms, about 0.05 mm. long, showing a dull brownish orange color with brown crossings under the microscope (Fig. 7); m. p. 97° (cor.). The solubility is similar to that of the U isomer; specific rotation, $[\alpha]^{25}_{\mathrm{Cd}}$ +365°, in petroleum ether.

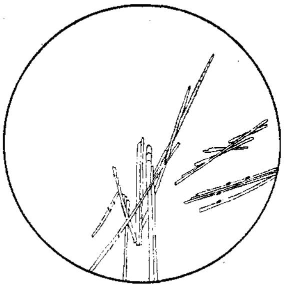

Fig. 7.—Neo-α-carotene W (from benzene and methanol).

(g) **Photo-isomerization.**—The petroleum ether solution (30 ml.) of 5 mg. of **all-*trans*-α-carotene** was exposed to bright sunlight in a transparent quartz test-tube (diameter, 22 mm.), filled with carbon dioxide, for forty-five minutes (final temperature, 29°). The colorimetric ratio was, unchanged all-*trans*:neo U:neo W:neo B:neo C... = 94:1.5:2:2:0.5.

A petroleum ether solution of 1 mg. of **neo-α-carotene** U in a quartz test-tube filled with carbon dioxide was exposed to bright sunlight for forty-five minutes and chromatographed (20 × 3.8 cm.).

4	orange, irreversible (could not be eluted)
15	almost colorless
45	light orange, neo U: 472.5, 441.5 mμ
4	colorless
5	yellow, neo V: 465.5, 438 mμ
7	colorless
14	pale orange, neo W: 470.5, 441.5 mμ
3	colorless
10	very pale orange, neo X: 463, 434.5 mμ
3	colorless
8	yellow, neo Y: 467.5, 437 mμ
3	colorless
15	dark orange, all-*trans*: 477, 446 mμ
3	colorless
10	very pale yellow, neo B...: 471.5, 442 mμ

The colorimetric ratio was, unchanged neo U:neo V:neo W:neo X:neo Y:all-*trans*:neo B... = 64.5:1.5:3.5:2:3:24:1.5.

In another experiment a homogeneous neo-α-carotene U solution was exposed at a distance of 10 cm. to the light of a 1000-watt Mazda bulb for forty-five minutes. A cold finger kept the temperature below 28.5°. The colorimetric ratio was, unchanged neo U:neo X:all-*trans* = 92:4.5:3.5. The light of an ultraviolet lamp (Hanovia, Luxor

Scientific Type) at 10 cm. distance isomerized only 2% of neo-α-carotene U in thirty minutes; some all-*trans* form, neo X, and neo Y appeared in the Tswett column. Solutions of the neo-α-carotenes V, W, and B were insolated for forty-five minutes. The colorimetric ratios are summarized in Table VII.

TABLE VII

RELATIVE COLORIMETRIC VALUES OF SOME MEMBERS OF THE α- AND β-CAROTENE SETS FORMED BY FORTY-FIVE MINUTES INSOLATION IN PETROLEUM ETHER SOLUTION

	Relative colorimetric values (% of the recovered pigment)								
Starting material	neo U	neo V	neo W	neo X	neo Y	all-*trans*	neo A	neo B	neo C...
				α-Carotene					
Neo U	64.5	1.5	3.5	2	3	24	—	1.5	—
Neo V	33	43	4	2	—	16	—	2	—
Neo W	7.5	5	32.5	1	—	41	4	7.5	1.5
All-*trans*	1.5	—	2	—	—	93.5	—	2.5	0.5
Neo B	1.5	—	34	—	—	56.5	—	8	—
				β-Carotene					
Neo U	36.5	—				55		6	2.5
All-*trans*	1	—				98		1	—
Neo B	27	2.5				60		5	5.5

(h) Sequence of Zones Obtained by Iodine Isomerization of a Mixture of α- and β-Carotene.—Since the two all-*trans* carotenes yield isomers with both increased and decreased adsorption affinities, a great number of zones overlap in a chromatogram. After catalysis the pigment mixture was developed on calcium hydroxide with petroleum ether containing 2% acetone. The chromatogram did not contain colorless sections; nevertheless, sections of different colors enabled the experimenter to cut out suitable zones. The addition of iodine to the solution of each pigment in the spectroscopic cell established the set to which the isomer belonged. This procedure was the basis for the chromatographic investigation of suitable binary mixtures. Such pairs were submitted to a prolonged development with petroleum ether. The sequence established in the order of decreasing adsorption affinity follows: neo-β-carotene U, neo-β-carotene V, neo-α-carotene U, all-*trans*-β-carotene, neo-α-carotene V, neo-β-carotene B, neo-β-carotene E, neo-α-carotene W, neo-β-carotene F, all-*trans*-α-carotene, neo-α-carotene B, and neo-α-carotenes C, D, etc.

Summary

Some data are given concerning *cis–trans* isomerization in the stereoisomeric α-carotene set as compared with the β-carotene, lutein, and lycopene sets. Ten *cis–trans* isomeric α-carotenes were observed which adsorb partly above, partly below the all-*trans* pigment in the Tswett column. Photo-isomerization experiments are described. The contribution of some stereoisomers to the *cis*-peak of the iodine equilibrium mixture was determined. Some spectral data served as a basis for a discussion of configurations.

PASADENA, CALIFORNIA RECEIVED AUGUST 2, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF OCCIDENTAL COLLEGE]

The Use of Potassium *t*-Amyloxide for the Alkylation of Acetoacetic Ester and its Alkyl Substitution Products

BY W. B. RENFROW, JR.

Alkylation of acetoacetic ester with sodium ethoxide in ethanol and an alkyl bromide gives yields of 70–80% with *n*-alkyl bromides,[1] but secondary alkyl bromides and bromides with a branch in the chain at the β-position (*i. e.*, isobutyl) generally give less than a 30% yield[2] of mono-substituted acetoacetic ester. Moreover, the further alkylation in the usual way of a mono-α-substituted acetoacetic ester with an *n*-alkyl bromide generally gives less than a 40% yield of disubstituted acetoacetic ester.[3]

Theoretical considerations indicated that the yields in the less favorable cases could be improved by the use of an alkoxide of greater proton-affinity than sodium ethoxide. Potassium *t*-amyloxide is a conveniently available base which is stronger than sodium ethoxide,[4] and was selected for investigation.

Table I gives the results obtained. The course of the reaction was followed by titration of aliquots with acid, and the approximate times were determined at the temperature of the refluxing solutions for one-half of the base to react.

Comparison of the data in Table I with similar data using sodium ethoxide[1,5,6] shows that potassium *t*-amyloxide in *t*-amyl alcohol and sodium ethoxide in ethanol are of about equal effectiveness for the alkylation of ethyl acetoacetate with primary, straight-chain bromides. However, potassium *t*-amyloxide gives considerably better yields of ethyl α-isopropylacetoacetate, ethyl α-isobutylacetoacetate, ethyl α,α-diethylacetoacetate and ethyl α,α-dibutylacetoacetate than can be obtained by use of sodium ethoxide in ethanol.[2,7,3]

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[1] For the usual method of alkylation, see: Gilman, Blatt, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, New York, N. Y., 1941, p. 248.

[2] In the present investigation ethyl isopropylacetoacetate and ethyl isobutylacetoacetate were obtained in 20–25% yields by the method described in (1).

[3] Hess and Bappert, *Ann.*, **441**, 151 (1925), obtained a 38% yield of α,α-di-*n*-butylacetoacetic ester under certain conditions. Under other conditions the product was almost exclusively ethyl di-*n*-butylacetate. Compare also Billon, *Ann. chim.*, **7**, 355 (1927).

[4] McEwen, THIS JOURNAL, **58**, 1124 (1936), found the following relative values for *pKa*: methanol, 16; ethanol, 18; *t*-amyl alcohol, 19.

[5] Michael, *J. prakt. Chem.*, **72**, 553 (1905).

[6] Locquin, *Bull. soc. chim.*, **31**, 758 (1904).

[7] Conrad and Limpach, *Ann.*, **192**, 153 (1878).