CONTRIBUTIONS TO THE STEREOCHEMISTRY OF CRYPTOXANTHIN AND ZEAXANTHIN

Thesis by

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Introduction

The phenomenon of carotenoid isomerization was detected for β-carotene ($C_{40}H_{56}$) by Gillam and El Ridi in 1935[1]. Since that time much work has been done, especially with β-carotene and lycopene, in detecting new stereoisomers and in correlating their structures with observed spectroscopic data[2,3,4,5,6]. It was at first suggested by Gillam and El Ridi[7] that the carotenoid isomers were formed as a result of double bond migration. This suggestion had to be abandoned in favor of the alternative explanation of trans-cis shifts due to the impossibility of accounting for all the observed stereoisomers of lycopene on the basis of double bond migrations[5]. Furthermore, lutein cannot be converted into zeaxanthin (which differs from lutein only in the position of one double bond) by treatment with iodine, which is an excellent catalyst for the conversion of one carotenoid stereoisomer into another by means of trans-cis shifts. Other arguments in favor of stereoisomerization have been presented based on the change in intensity of light absorption[8] and the spectral shift[3] upon the production of one stereoisomer from another. Consequently, cis-trans isomerization about the conjugated double bonds of carotenoids must be accepted as the correct explanation of carotenoid isomerization.

The purpose of the present work is to contribute to the knowledge of the stereoisomerization of cryptoxanthin ($C_{40}H_{55}OH$) and zeaxanthin ($OH \cdot C_{40}H_{54} \cdot OH$), which are, respectively, mono- and di-hydroxy β-carotene. The following principal methods were used to promote the isomerization, viz., (1) iodine catalysis at room temperature, (2) melting the crystals, (3) refluxing the pigment solution, and (4) irradiation. The stereoisomers

obtained were separated by means of the Tswett chromatographic method, which is, indeed, the only means available for such separations. The identification of the stereoisomers is accomplished spectroscopically and, in some cases, by mixed chromatograms.

The earlier assumption[9] that carotenes and carotenoids with only one free hydroxyl group yield stereoisomers which are adsorbed at lower sections of the Tswett column than the all-trans stereoisomer was modified as a result of the work of Polgár and Zechmeister[2] on β-carotene isomerization, during which three new isomers were found which were adsorbed above the all-trans compound. A stereoisomer of cryptoxanthin was detected during the present work which is also adsorbed above all-trans cryptoxanthin on the Tswett column. In addition, two new stereoisomers of zeaxanthin are reported in this work which are adsorbed below the all-trans stereoisomer. All previously reported zeaxanthin isomers were absorbed at higher sections of the column than the natural (all-trans) zeaxanthin.

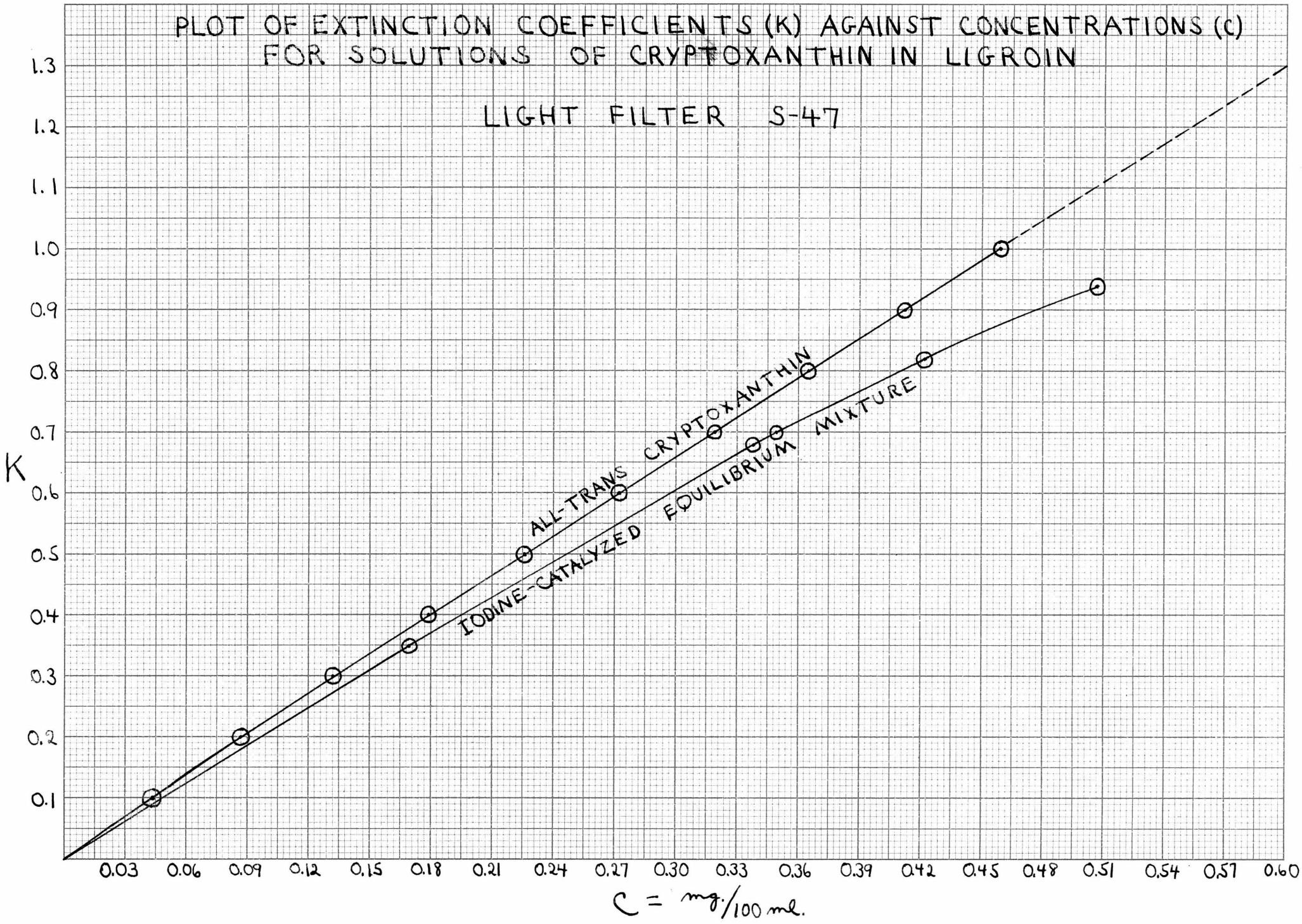
Included in the present work is an investigation into the absorption of light in the ultra-violet region of the spectrum by some cryptoxanthin stereoisomers. The interesting phenomenon of the formation of "cis-peaks" in this region by several carotenes has recently been described[10]. An analogous behavior has been observed during this work in the case of cryptoxanthin. It has already been pointed out[10] that the height of "cis-peaks" may depend on the position of each cis double bond in the long polyenic chain of the carotenoids. This subject will later be discussed for the special case of cryptoxanthin.

Experimental

I. Cryptoxanthin

Methods. The cryptoxanthin used in the following work was obtained in Hungary from the red berries of Physalis Alkekengi by extraction of the milled berries with ether followed by saponification with methanolic potassium hydroxide. The samples were purified chromatographically and kept in crystalline form under an atmosphere of carbon dioxide in sealed tubes.

The pigment solutions were chromatographed on calcium hydroxide (Shell brand lime, chemical hydrate). Petroleum ether (b.p. 60-70°) containing 5-15% acetone was used for development. The pigments were eluted from the columns with alcohol using sintered glass funnels (Jena 11G3 to 26G3), transferred into petroleum ether and washed free of alcohol in the apparatus described by LeRosen[11]. All spectra were taken in petroleum ether and were determined with an Evaluating Grating Spectroscope (Zeiss, light filter BG-7, 2mm. thick). Concentrations of solutions were estimated by means of a Pulfrich Gradation Photometer (light filter S47). Photometric values for the naturally occurring all-trans cryptoxanthin were published by Cholnoky[12]. Since approximately the same equilibrium mixture of stereoisomers is always obtained on addition of iodine to any cis-trans isomer (of a given carotenoid), the concentrations of the crypotxanthin stereoisomers were estimated by first adding iodine to the solution and then reading the light extinction coefficient in the Pulfrich Photometer. From this coefficient the pigments concentration can be determined from the data which is shown graphically below. This data was obtained by adding iodine to cryptoxanthin solutions of known concentrations in petroleum ether and determining the extinction coefficients of these solutions in the Photometer.


PLOT OF EXTINCTION COEFFICIENTS (K) AGAINST CONCENTRATIONS (C)
FOR SOLUTIONS OF CRYPTOXANTHIN IN LIGROIN
LIGHT FILTER S-47
K
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ALL-TRANS CRYPTOXANTHIN
IODINE-CATALYZED EQUILIBRIUM MIXTURE
0.03 0.06 0.09 0.12 0.15 0.18 0.21 0.24 0.27 0.30 0.33 0.36 0.39 0.42 0.45 0.48 0.51 0.54 0.57 0.60
C = mg./100 ml.

In the descriptions of chromatograms to follow, the figures on the left side of the described chromatograms denote width of the zones in mm. The figures on the right of the described zones are the extinction maxima (both before and after adding iodine) of the pigment in that particular zone in milli-microns. The extinction maxima figures after addition of iodine are underlined.

(a) Isomerization of Cryptoxanthin by Iodine Catalysis at Room Temperature. 4.6 mg. of chromatographically homogeneous cryptoxanthin was dissolved in 30 ml. of petroleum ether. To this solution was added 0.06 mg. of iodine in the same solvent and the solution was allowed to stand at room temperature for sixty minutes. It was then adsorbed on calcium hydroxide (20 x 3.5 cm.) giving the following chromatogram:

2	brownish yellow: heterogeneous irreversible layer
110	colorless
12	yellowish orange: neo-cryptoxanthin U (478.5, 448 mμ), (480, 449 mμ)
2	almost colorless
26	orange: all-trans-cryptoxanthin (483.5, 452.5), (480, 448.5)
2	colorless
10	yellow: neo-cryptoxanthin A (477, 446), (480, 448.5)
7	brownish yellow: neo-cryptoxanthin B (479.5, 449.5), (480, 448)

The unchanged cryptoxanthin and the three stereoisomers were cut out separately, eluted, transferred into petroleum ether and washed free of alcohol. Each stereoisomer was again submitted to the same catalytic treatment and again chromatographed. The relative photometric values of the isomers formed are summarized in Table I.

Table I

Relative Photometric Values of Cryptoxanthin and

of Some of its Stereoisomers as Formed by Iodine Catalysis

at Room temperature

Starting Material	Relative Photometric Values (%)			
	Neo U	Cryptoxanthin	Neo A	Neo B
Neo-cryptoxanthin U	23	56	22	*
All-trans-cryptoxanthin	18	59	18	5
Neo-cryptoxanthin A	20	57	23.	*
Neo-cryptoxanthin B	21	55	17	7

* None visible on column

(b) Isomerization of Cryptoxanthin by Melting

This experiment was carried out by sealing crystals of chromatographically homogeneous cryptoxanthin in a glass tube under carbon dioxide. Such tubes were immersed in a bath at 170° for varying periods of time, viz., 2, 8, 10 and 15 minutes. The melt was then rapidly solidified in ice-water, dissolved in petroleum ether and chromatographed. The melts for two and eight minutes produced only one stereoisomer which was absorbed immediately below unchanged cryptoxanthin on the column. It was identified spectroscopically with "neo-cryptoxanthin"[5] and termed neo-cryptoxanthin A. The ten minutes melting produced an amount of a neo isomer, ~~of~~ neo-cryptoxanthin U, which was equal to 22% of the total pigment of the column. 30% of the pigment was neo-cryptoxanthin A and the remainder unchanged cryptoxanthin. The melt for fifteen minutes was carried out with 3.2 mg. of cryptoxanthin and gave the following chromatogram (18 x 1.8 cm.):

1 brownish yellow: irreversible layer
75 colorless
15 yellowish orange: neo-cryptoxanthin U (478, 448 mμ), (497.5, 449 mμ)
28 orange: all-trans-cryptoxanthin (483.5, 452.5), (480.5, 449)
19 yellow: neo-cryptoxanthin A (476, 446), (480, 449)
1 colorless
6 yellowish orange: neo-cryptoxanthin B (479, 448.5), (480.5, 449)

The neo-cryptoxanthin U obtained by melting was shown by means of a mixed chromatogram to be identical with the corresponding zone formed by iodine catalysis.

The relative amounts of the stereoisomers separated on the above chromatogram are listed in Table II. The total loss in pigment during the melt was 55%.

Table II

Relative Amounts of the Stereoisomers Formed on Melting Crystals of Cryptoxanthin

Stereoisomer	% of Total Pigment After Melting
Neo-cryptoxanthin U	7
All-trans-cryptoxanthin	49
Neo-cryptoxanthin A	38
Neo-cryptoxanthin B	6

In addition to the above melts, a lower temperature melt of cryptoxanthin was carried out by thoroughly mixing 2.0 mg. of chromatographically homogeneous cryptoxanthin with 8.5 mg. of naphthalene in a sealed tube under carbon dioxide, placing the tube in a bath at 115° for five minutes and finally quickly cooling by plunging into a bath of ice water. The contents of the tube was dissolved in 20 ml. of petroleum ether and chromatographed. The following chromatogram (18 x 1.8 cm.) was obtained:

2 yellowish brown: irreversible layer
52 colorless
36 orange: all-trans-cryptoxanthin (483.5, 452.5 mμ), (480, 450 mμ)
18 yellowish orange: neo-cryptoxanthin A (478, 447), (480.5, 449.5)
8 pale yellowish brown: neo-cryptoxanthin B (480, 450.5), (480, 449.5)

In order to confirm the absence of neo-cryptoxanthin U, the top fifth of the all-trans cryptoxanthin zone was cut out and eluted separately. It was found to have a clear all-trans cryptoxanthin spectrum with absorption maxima at 483.5 and 452.5 mμ.

(c) Heat Isomerization of Cryptoxanthin Solutions

1. Refluxing at 60°.

0.58 mg. (value determined photometrically) of chromatographically homogeneous cryptoxanthin in 100 ml. of petroleum ether (b.p. 60-70°) was refluxed in an all-glass apparatus in a slow stream of carbon dioxide for one hour. The following chromatogram was obtained (18 x 1.8 cm.):

5 pale yellow: irreversible layer
74 colorless
22 orange: all-trans-cryptoxanthin (484.5, 453.5), (480.5, 450)
1 colorless
9 yellow: neo-cryptoxanthin A (477, 446), (480, 449.5)

The spectrum of the top fifth of the orange layer gave a sharp cryptoxanthin spectrum indicating the absence of any neo-cryptoxanthin U.

A solution of homogeneous neo-cryptoxanthin A was refluxed for one hour under the same conditions as above. From the solution a chromatogram very similar to the one above was obtained, i.e., only cryptoxanthin and neo-cryptoxanthin A were present on the column. Some photometric values are given in Table III.

Table III

Amounts of Cryptoxanthin and Neo-cryptoxanthin Present After Refluxing a Pure Solution of Each For Sixty Minutes at 60-70°

Starting Material	% of Total Pigment After Refluxing	
	Cryptoxanthin	Neo-cryptoxanthin A
All-trans-cryptoxanthin	88	12
Neo-cryptoxanthin A	64	36

2. Refluxing at 120°

6.2 mg. of chromatographically homogeneous cryptoxanthin were dissolved in 50 ml. of ligroin (b.p. 120°) and refluxed for thirty minutes in an all-glass apparatus in a slow stream of carbon dioxide. The following chromatogram was obtained (20 x 3.5 cm.):

5 yellow: irreversible zone
64 colorless
23 orange: all-trans-cryptoxanthin (483.5, 452.5 mμ), (480, 449 mμ)
2 colorless
14 yellowish orange: neo-cryptoxanthin A (477, 446), (480, 449)
12 yellow: neo-cryptoxanthin B (479.5, 447.5), (480, 449.5)

A spectroscopic check on the upper fifth of the all-trans cryptoxanthin layer indicated the absence of any neo-cryptoxanthin U. The pigment fractions had the relative photometric values given in Table IV.

Table IV

Photometric Values of the Stereoisomers Formed by Refluxing Cryptoxanthin for Thirty Minutes at 120°

Stereoisomer	% of Total Extinction
All-trans-cryptoxanthin	62
Neo-cryptoxanthin A	32
Neo-cryptoxanthin B	6

(d) Isomerization of Cryptoxanthin by Irradiation.

1. Irradiation by Quartz Lamp.

Three solutions of chromatographically pure cryptoxanthin each containing 0.225 mg. in 20 ml. of petroleum ether (b.p. 60-70°) were exposed in quartz tubes to the ultra violet light of a quartz lamp (Hanovia, Luxor Scientific type) for five, fifteen and thirty minutes. The distance from the lamp to the tubes was approximately 15 cm. The solutions were then chromatographed on calcium hydroxide. Except for a small irreversible layer at the very top of the columns, only unchanged cryptoxanthin appeared in all cases. However, the top fifth of the cryptoxanthin zone obtained after thirty minutes irradiation gave a spectrum with absorption maxima at 481 and 449.5 mμ. This indicates the formation of a small amount of neo-cryptoxanthin U in this case.

2. Irradiation by Direct Sunlight.

2.0 mg. of chromatographically homogeneous cryptoxanthin was dissolved in 30 ml. of petroleum ether (b.p. 60-70°). This solution was placed in a quartz flask filled with carbon dioxide and exposed to direct sunlight for forty-five minutes. The temperature of the solution at the end of this time was 34°. The following chromatogram was obtained (18 x 1.8 cm.):

4 yellowish brown: irreversible pigment
83 colorless
26 orange: all-trans-cryptoxanthin (483.5, 453 mμ), (480, 449.5 mμ)
13 yellow: neo-cryptoxanthin A (478, 446.5), (479.5, 448.5)

Neo-cryptoxanthin A was the only stereoisomer obtained on insolation. It amounted to 14% of the total pigment on the column. The upper fifth of the orange cryptoxanthin zone was examined in the spectroscope but showed only a sharp all-trans cryptoxanthin spectrum. Apparently there is no neo-cryptoxanthin U formed on exposure of cryptoxanthin to sunlight.

(e) Determination of the Molecular Extinction Curves for All-trans-Cryptoxanthin, Neo-cryptoxanthin U, and Neo-Cryptoxanthin A, Neo-cryptoxanthin B and the Cryptoxanthin Iodine Equilibrium Mixture in the Region 320 to 380 mμ.

All spectrophotometric readings were taken in "Hexane" (from petroleum, practical, Eastmen-Kodak Co.) which was purified by mechanically shaking twice with fuming sulfuric acid for one hour and washing acid free with water. The fraction between 62-66° was used for spectrophotometric purposes. The quantitative readings were taken in a Beckman quartz photoelectric spectrophotometer[13].

The stereoisomers used for these determinations were prepared by melt isomerization of cryptoxanthin. The cryptoxanthin, neo-cryptoxanthin U, neo-cryptoxanthin A and neo-cryptoxanthin B zones were cut out of the chromatogram, eluted with alcohol and transferred into hexane by means of ice-cold

water. Each solution was washed alcohol free and divided into two parts. One part was made up to a suitable volume with pure hexane and the other with a solution of iodine in hexane. During the following hour the readings were taken on the spectrophotometer. The four iodine solutions were used to determine the molecular extinction curve of the iodine equilibrium mixture. The average of these four curves is the iodine equilibrium curve which appears, together with the curves of the individual stereoisomers, on the following page. The blanks used for the spectrophotometric readings were either pure hexane or an iodine solution in hexane of a corresponding concentration.

The molecular extinction coefficients were calculated as follows:

$$E_{1cm}^{mol} = \varepsilon = \log \frac{I_o}{I} \cdot \frac{1}{Cm}$$

I_o = light transmission by pure solvent
I = " " " solution
Cm = molecular concentration

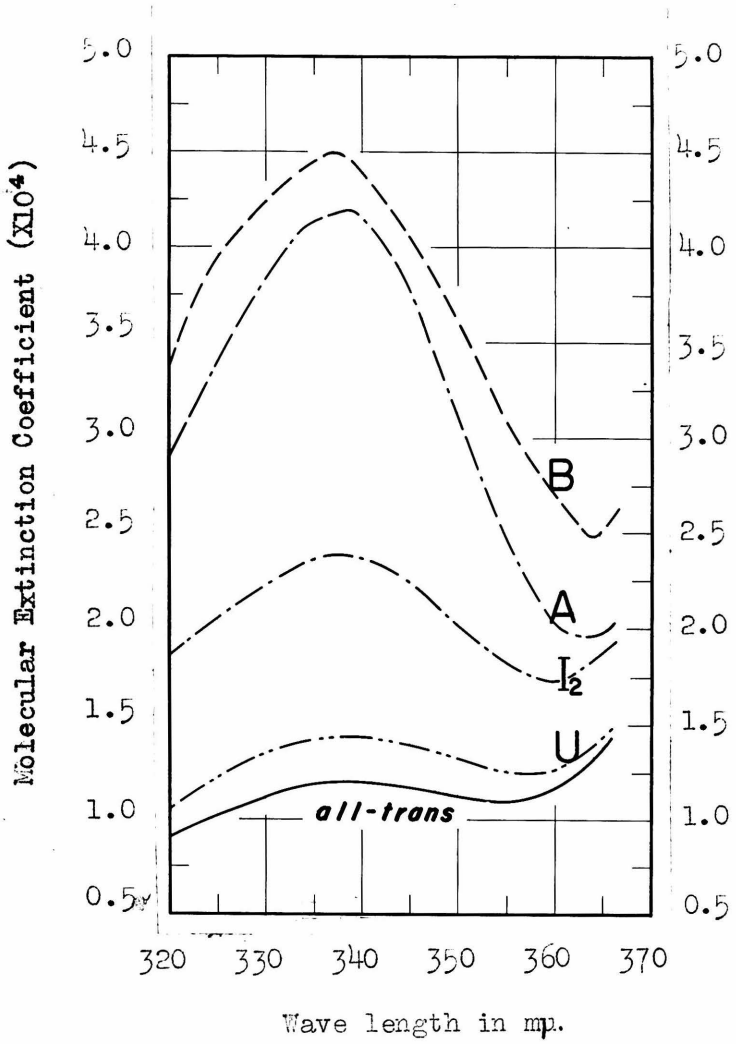


Figure 1

Molecular extinction curves of cryptoxanthin stereoisimers; contribution of the four main constituents of the stereoisomeric mixture to the cis-peak observed on addition of iodine; ——— all-trans-cryptoxanthin, -.-.-. neo-cryptoxanthin U, __.__. mixture after iodine catalysis, --.--. neo-cryptoxanthin A, __ __ __ __ neo-cryptoxanthin B.

II. Zeaxanthin

Methods. The zeaxanthin used in the following work originated from the red berries of Phsalis Alkekengi by extraction of the milled berries with ether followed by saponification with methanolic potassium hydroxide. The zeaxanthin was purified chromatographically and kept ready for use during the course of the investigation in crystalline form in a sealed tube under an atmosphere of carbon dioxide.

The pigment solutions were chromatographed on calcium carbonate (Merck's Heavy Powder) or, in cases where a somewhat stronger adsobbent was required, on a 1:1 mixture of calcium carbonate and hydroxide. Pure benzene or benzene containing up to 50% petroleum ether was used for development. The pigments were eluted from the columns with alcohol on sintered glass funnels (Jena 11G3 to 26G3), transferred into benzene and washed free of alcohol in the apparatus described by LeRosen[11]. All spectra were taken in benzene and were determined with an Evaluating Grating Spectroscope (Zeiss, light filter BG-7, 2 mm. thick). Concentrations of solutions were estimated by means of a Pulfrich Gradation Photometer (light filter S47). Photometric values for zeaxanthin were published by Cholnoky[12].

In the descriptions of chromatograms to follow, the figures on the left side of the described chromatograms denote width of the zones in mm. The figures on the right of the described zones are the extinction maxima (both before and after adding iodine) of the pigment in that particular zone in milli-microns. The absorption maxima figures after adding iodine are underlined.

(a) Isomerization of Zeaxanthin by Melting.

3.2 mg. of chromatographically homogeneous zeaxanthin crystals was sealed in a small glass tube under carbon dioxide and kept in a bath at 220° for fifteen minutes. The loss of colorimetric intensity was so great that not enough of any stereoisomer could be separated on the chromatographic column to permit reading its spectrum.

5.2 mg. of chromatographically homogeneous zeaxanthin crystals was sealed with 21.6 mg. of naphthalene under carbon dioxide in a small tube. The latter was kept in a bath at 160° for ten minutes after which it was quickly cooled in ice water. The contents of the tube was then dissolved in 30 ml. of benzene. This solution gave the following chromatogram (18 x 1.8 cm.):

Section I	3	yellow: irreversible layer
	5	yellowish orange
	2	almost colorless
	5	yellowish orange
	2	almost colorless
	8	yellow
Section II	6	colorless
	94	yellow

Sections I and II were rechromatographed.

Section I gave the following chromatogram (18 x 1.8 cm.):

22	colorless
5	yellowish orange: neo-zeaxanthin A (489.5, 458 mμ), (492.5, 460.5)
3	colorless
6	yellowish orange: neo-zeaxanthin B1 (489.5, 457.5), (493.5, 460.5)
3	colorless
3	yellowish orange: neo-zeaxanthin B2 (489.5, 457.5), (493, 460)
4	colorless
4	yellow: neo-zeaxanthin C (485.5, 454.5), (493, 460)

Section II gave the following chromatogram (18 x 1.8 cm.):

5 colorless
4 yellowish orange: neo-zeaxanthin A (490, 458.5), (492.5, 460)
8 colorless
4 yellowish orange: neo-zeaxanthin B1 (490, 458), (492.5, 460)
5 colorless
11 yellowish orange: all-trans-zeaxanthin (494.5, 462), (492.5, 460)
2 colorless
3 pale yellow: unknown pigment (not enough for spectrum)

The identities of the neo-zeaxanthin A and neo-zeaxanthin B above were established by mixed chromatograms with previously obtained neo A and neo B stereoisomers. They were apparently formed by spontaneous isomerization while the lower section of the first chromatogram stood in solution at room temperature.

(b) Heat Isomerization of Zeaxanthin.

1. Refluxing at 60°.

10.0 mg. of chromatographically homogeneous zeaxanthin was dissolved in 80 ml. of petroleum ether (b.p. 60-70°) and refluxed in an all-glass apparatus in a stream of carbon dioxide for thirty minutes. The following chromatogram was obtained (20 x 3.5 cm.):

8 pale yellow: irreversible layer
3 yellowish orange: neo-zeaxanthin A (489.5, 457.5 mμ), (492.5, 460 mμ)
3 colorless
2 yellowish orange: neo-zeaxanthin B1 (490, 457.5), (493, 460)
2 colorless
4 yellow: neo-zeaxanthin B2 (489.5, 457.5), (493, 459.5)
43 almost colorless
20 orange: all-trans zeaxanthin (494, 462), (493.5, 459.5)

2. Refluxing at 125°.

10.5 mg. of chromatographically homogeneous zeaxanthin was dissolved in 80 ml. of ligroin (b.p. 125°) and refluxed in an all-glass apparatus in a stream of carbon dioxide for thirty minutes. The following chromatogram was obtained (20 x 3.5 cm.):

6 pale yellow: irreversible layer
6 orange: neo-zeaxanthin A (489.5, 457.5 mμ), (493, 460 mμ)
3 almost colorless
4 orange: neo-zeaxanthin B1 (490.5, 458), (493, 459.5)
10 almost colorless
12 pale orange: neo-zeaxanthin B2 (489.5, 457.5), (493.5, 459.5)
15 colorless
7 orange: all-trans-zeaxanthin (494, 462), (493.5, 459.5)
19 colorless
6 yellowish orange: neo-zeaxanthin S (491.5, 458.5), (493.5, 460)

The relative photometric values of the above stereoisomers are given in Table V.

Table V

Photometric Values of the Stereoisomers Formed by Refluxing Zeaxanthin for Thirty Minutes at 125°

Stereoisomer	% of Total Extinction
Neo-zeaxanthin A	22
Neo-zeaxanthin B1	17
Neo-zeaxanthin B2	21
All-trans-zeaxanthin	35
Neo-zeaxanthin S	5

(c) Irradiation of Zeaxanthin

1. Irradiation by Quartz Lamp

Three solutions of 0.9 mg. of chromatographically homogeneous zeaxanthin dissolved in 25 ml. of benzene were exposed to the ultra-violet light of a quartz lamp (Hanovia, Luxor Scientific type) for the respective periods of five, fifteen and thirty minutes. The shorter irradiation gave solutions which showed, except for a small irreversible layer at the top, only unchanged zeaxanthin on the chromatographic column. However, the sample irradiated for thirty minutes gave the following chromatogram (18 x 1.8 cm.):

3 pale yellow: irreversible layer
15 colorless
1 orange-brown: unknown pigment (not enough for spectrum)
40 colorless
12 yellowish orange: neo-zeaxanthin A (490.5, 459.5 mμ), (493, 460)
18 colorless
24 orange: all-trans-zeaxanthin (494, 459), (493, 460)

The relative photometric values of the stereoisomers were as follows:

Unknown pigment	5%
Neo-zeaxanthin A	35%
Zeaxanthin	60%

2. Irradiation by Sunlight

10 mg. of chromatographically homogeneous zeaxanthin was dissolved in 50 ml. of benzene and exposed in a quartz tube to bright sunlight for fifteen minutes. The temperature of the solution at the end of this time was 31°. The solution gave the following chromatogram (20 x 3.5 cm.):

6 pale yellow: irreversible layer
2 yellowish orange: neo-zeaxanthin A (490, 459 mμ), (492.5, 459.5 mμ)
2 colorless
2 yellowish orange: neo-zeaxanthin B (489.5, 459), (492, 459.5)
40 almost colorless
13 orange: all-trans-zeaxanthin (493.5, 462), (492.5, 460)
42 pale yellow

The bottom layer gave on rechromatography the following zones:

20 colorless
8 yellowish orange: neo-zeaxanthin S (490.5, 457.5), (492.5, 459)
3 colorless
9 yellowish orange: neo-zeaxanthin T (490, 457), (492, 459)

The relative photometric values of the stereoisomers formed on insolation are given below in Table VI.

Table VI

Photometric Values of the Stereoisomers Formed by the Insolation of Zeaxanthin for ~~Thirty~~ FIFTEEN Minutes

Stereoisomer	% of Total Extinction
Neo-zeaxanthin A	16
Neo-zeaxanthin B	11
All-trans-zeaxanthin	65
Neo-zeaxanthin S	5
Neo-zeaxanthin T	3

Zeaxanthin was also insolated for periods of five and thirty minutes. However, it was found that five minutes insolation produced very little stereoisomerization while thirty minutes insolation resulted in almost total destruction of the pigment.

Discussion

I. Cryptoxanthin

The described isomerizations of cryptoxanthin produced two new stereoisomers, namely, neo-cryptoxanthin U and neo-cryptoxanthin B*. The detection of neo-cryptoxanthin A has been previously reported under the name of "neo-cryptoxanthin"[5,6,14]. The best method for preparing the two new stereoisomers appears to be by the melt isomerization. As a melt isomerization of cryptoxanthin has never been previously attempted, it is not surprising that new stereoisomers appeared after such treatment. The new stereoisomers can also be detected on the Tswett column after iodine catalysis, in fact, iodine catalysis gives a larger proportion of the U isomer than does a melt isomerization. However, during the course of this investigation neo-cryptoxanthin B was reliably and consistently produced on melt isomerization whereas it was often difficult to effect its chromatographic separation after iodine isomerization.

The use of calcium hydroxide as adsorbent and petroleum ether containing small percentages of acetone as developer seems to be an excellent system for the chromatographing of the cryptoxanthin set of stereoisomers. This may be the reason why new stereoisomers have been observed in the course of this work which were not detected in previous work on cryptoxanthin using calcium hydroxide and benzene-petroleum ether mixtures[5,6] or using calcium hydroxide and developing with a hexane-ether mixture[14]. The acetone has the additional advantage that it can be washed out of the petroleum ether by water. Thus the pigment may be quantitatively transferred into petroleum

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* This nomenclature is used to conform to that which was applied to the stereoisomers of β-carotene in the recent work of Polgár and Zechmeister[2].

ether for spectroscopic or photometric measurements without previous evaporation.

The ideal temperature for and time of duration of cryptoxanthin melt isomerizations ~~is~~ are yet to be determined. It is obvious that the higher the temperature and longer the time of melting, the greater must be the destruction of the pigment through oxidation and pyrolysis. At the same time, however, higher temperatures have a marked tendency to promote stereoisomerization. We notice, therefore, a competition between the processes of destruction and stereoisomerization and it is not yet determined quantitatively where in the range of time and temperature is the point at which the net production of stereoisomers is at an optimum. The purpose of the addition of naphthalene is to decrease the temperature at which the melt isomerization may be carried out. Such melts resulted in far less destruction of the pigment but also resulted in lower yields of stereoisomers.

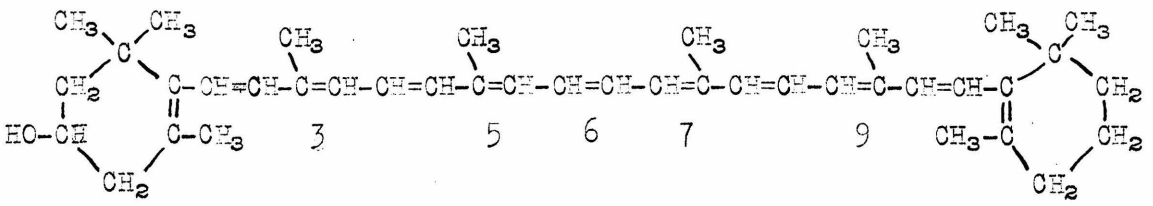
An intermediate stage between a naphthalene melt and a refluxing at 60-70°[2] is a refluxing of the pigment at a considerably higher temperature, say 120°. Such a high boiling reflux was carried out and gave results which did not vary appreciably from those obtained by the naphthalene melt.

Insolation seems to be a very promising method with which to induce the isomerization of carotenoids, particularly because of the very strong extinction of light in certain regions of the visible spectrum. In the insolation no less than in the heating of cryptoxanthin there is a manifest competition between the destruction and stereoisomerism of the carotenoid molecule. The described insolation experiment produced only neo-cryptoxanthin A but it is probable that a more prolonged (or even shortened) insolation would produce other stereoisomers too. Ultra-violet light alone has no

marked effect on cryptoxanthin, but this fact is not surprising in view of the relatively small light absorption by cryptoxanthin in the ultra-violet region.

Perhaps the most interesting part of the present work was the determination by means of the Beckman Spectrophotometer of the molecular extinction curves for some cryptoxanthin stereoisomers as well as the curve for the iodine equilibrium mixture. Such curves for cryptoxanthin and neo-cryptoxanthin (the neo-cryptoxanthin A of this work) have been reported by White, Zscheile and Brunson[14] for the visible range of the spectrum where the two large maxima of light absorption occur, namely, from 380 to 560 mμ. In view of the recent papers[10,15] which described the interesting phenomenon of the "cis-peak" in the region from 320 to 380 mμ, it was decided to check the behavior of the known cryptoxanthin stereoisomers in this region. The photograph of these curves on page 11 shows the position of the cis-peak for the iodine equilibrium mixture to be at approximately 338 mμ. The greatest contributions to this cis-peak are from the neo-cryptoxanthin A and B. Neo-cryptoxanthin U makes only a very small contribution and the all-trans form makes practically no contribution whatever. The corresponding β-carotene stereoisomers make exactly the same qualitative contribution to the β-carotene cis-peak[10].

It has been suggested[10] that the cis-peak effect is more dependent on the position of cis double bonds in the molecule than merely on the number of such bonds. For example, it is probable that the double bond in the carotenoid molecule which would be most readily converted into a cis configuration is the one in the center of the molecule. This bond is bond no. 6 in the structural formula of cryptoxanthin:



Cryptoxanthin. (The stereochemically effective double bonds are numbered.)

Double bond no. 6 would be subject to no possible steric hinderences and therefore is probably the most easily converted into a cis double bond. Consequently, the most easily produced stereoisomer of cryptoxanthin, namely, neo-cryptoxanthin A, probably results from the trans-cis-rotation of this bond. Furthermore, Dr. Pauling has suggested that such a cis double bond in this position in the carotenoid molecule, with its resultant bending of the polyenic chain in the center, would give rise to the largest dipole moment possible for the particular mode of electron oscillation up and down the polyenic chain which is associated with the absorption of light in the cis-peak region. This largest dipole moment would, in turn, give rise to the greatest absorption of light in this region, i.e. highest cis-peak. This idea seems to apply somewhat successfully to the cryptoxanthin stereoisomers since neo-cryptoxanthin A has quite a high cis-peak. However, neo-cryptoxanthin B, which is not as readily produced as neo A, has a similar or even slightly higher cis-peak. Since neo A has lower absorption maxima in the visible range of the spectrum than has neo B, neo A may actually contain two cis double bonds in accordance with the view that each cis double bond shifts the absorption maxima to shorter wave-lengths[3]. If this were the situation, neo B, with one cis double bond at 5 and 7, would be expected to give rise to a higher cis-peak than neo A with two cis bonds. However, with only four cryptoxanthin cis-trans isomers detected out of a possible thirty-two, much work remains to be done before the different stereoisomers will be correlated to definite steric structures.

II. Zeaxanthin

The isomerization of zeaxanthin by melting the crystals appears to be an unsuitable method due to the great amount of thermal destruction. A more successful method is to melt the crystals at lower temperatures by adding naphthalene. By means of the latter method, four stereoisomers of zeaxanthin were separated on the column, all above the all-trans-zeaxanthin zone. These were named, from top to bottom, neo A, neo B1, neo B2 and neo C. Neo-zeaxanthin A and B1 are apparently identical with the stereoisomers reported earlier under the names of neo A and neo B[9]. Neo B2 is a new stereoisomer while neo C is identical with the earlier reported neo C[9]. The best adsorbent for the separation of these stereoisomers is calcium carbonate. Mixtures of calcium carbonate with calcium hydroxide were unsuccessful, since only incomplete separations could be achieved with such a mixture. Benzene containing an adequate admixture of petroleum ether is a suitable developer. In contrast to the situation in the case of cryptoxanthin, petroleum ether cannot be used successfully for the spectroscopic and photometric characterizations of zeaxanthin and its stereoisomers due to their relative insolubility in this solvent. Benzene, therefore, was used as the solvent for all such characterizations.

The most successful isomerization of zeaxanthin was achieved by refluxing the pigment at a temperature of 125°. By means of this method, a new stereoisomer appeared which exhibited smaller adsorbability on the chromatographic column than the all-trans form. All other known stereoisomers possess stronger adsorption affinities than the all-trans form. This stereoisomer was called neo-zeaxanthin S (S for sub).

Although ultraviolet irradiation of zeaxanthin by means of a quartz lamp produced only neo-zeaxanthin A, insolation of zeaxanthin led to the detection of a further stereoisomer with an adsorption affinity less than

all-trans zeaxanthin. This stereoisomer appeared on the Tswett column immediately below neo-zeaxanthin S and was termed neo-zeaxanthin T.

Because of their greater sensitivity, zeaxanthin solutions cannot be exposed to sunlight as long as can cryptoxanthin solutions without causing considerable loss of pigment. However, an insolation for about fifteen minutes gives reasonably good results without undue destruction.

Summary

1. The stereoisomerization of cryptoxanthin was studied under the influence of iodine catalysis at room temperature, melting the crystals, refluxing and irradiation. Two new stereoisomers of cryptoxanthin were detected, one of which, neo-cryptoxanthin U, is adsorbed above all-trans-cryptoxanthin on the Tswett column.

2. Molecular extinction curves were taken in the region from 320 to 380 mμ. for all-trans-cryptoxanthin, three of its stereoisomers, and the cryptoxanthin iodine-catalyzed equilibrium mixture of stereoisomers.

3. The stereoisomerization of zeaxanthin was studied under the influence of melting the crystals, refluxing and irradiation. Three new stereoisomers of zeaxanthin were detected, two of which appear below all-trans-zeaxanthin on the Tswett column.

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