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Contribution to the Stereochemistry of Cryptoxanthin and Zeaxanthin

BY L. ZECHMEISTER AND R. M. LEMMON

Although the *cis–trans* isomerization of β-carotene has been studied in detail,[1] the investigation of its hydroxy derivatives, cryptoxanthin, $C_{40}H_{55}\cdot OH$, and zeaxanthin, $HO\cdot C_{40}H_{54}\cdot OH$, is limited. Only those stereoisomerization phenomena which result from the iodine catalysis or heating of solutions have been studied.[2] Those spectral alterations which are caused by the bending of the molecule and take place in a certain section of the ultraviolet region[3,4] have not yet been examined.

In earlier experiments only one stereoisomer of natural (all-*trans*-) cryptoxanthin, *viz.*, "neocryptoxanthin," was reported. Three stereoisomers of natural zeaxanthin, the neo-forms A, B and C, were studied more extensively. In contrast to "neocryptoxanthin," the neozeaxanthins were found to possess a stronger adsorption affinity than the all-*trans* compound. The present reinvestigation of the stereoisomerization of zeaxanthin by methods which among others included melting of crystals and refluxing in higher boiling solvents has revealed no new members of this stereoisomeric set. However, two new isomers of cryptoxanthin were observed and named in accordance with analogous members of the β-carotene set. The minor isomer, neo B, is adsorbed on the Tswett column below "neocryptoxanthin" (now termed neo A). Neocryptoxanthin U which is adsorbed above the all-*trans* pigment appears upon careful development with petroleum ether–acetone mixtures. Its characteristics in many respects are similar to those of neo-β-carotene U[1] and neo-α-carotene U,[4] which likewise possess increased adsorbability relative to their all-*trans* forms.

In the present paper we wish also to report on the changes which occur in dilute solutions of cryptoxanthin and zeaxanthin (1 to 10 mg. per 100 ml.) during exposure to intense sunshine.[5] Under such conditions a gradual bleaching takes place, the rate of which for some reason is considerably greater for zeaxanthin than for cryptoxanthin (Table I).

TABLE I

LOSS OF COLOR INTENSITY OF BENZENE SOLUTIONS OF CRYPTOXANTHIN AND ZEAXANTHIN ON EXPOSURE TO INTENSE SUNSHINE

Duration of insolation, min.	Loss of color intensity (% of the initial value) Cryptoxanthin	Zeaxanthin
15	6	21
30	17	39
45	26	63
60	36	88

This loss in colorimetric intensity is a result of three processes: first, stereoisomerization; second, structural conversion to other pigments; and third, cleavage to colorless or nearly colorless substances. A short exposure to sunshine causes a small amount of *trans*→*cis* isomerization. While this rearrangement is taking place, the other reactions begin. Finally, the cleavage becomes the predominant process. Therefore, the proper duration of the insolation must be established for each carotenoid in order to study its photoisomerization.

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

(2) L. Zechmeister and P. Tuzson, *Biochem. J.*, **32**, 1305 (1938); *Ber.*, **72**, 1340 (1939); L. Zechmeister, L. Cholnoky and A. Polgár, *ibid.*, **72**, 1678 and 2039 (1939); H. H. Strain, "Leaf Xanthophylls," Washington, 1938.

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

(4) L. Zechmeister and A. Polgár, *ibid.*, **66**, 137 (1944).

(5) Insolation experiments have been reported for α-carotene, β-carotene, prolycopene, pro-γ-carotene, and capsanthin. *Cf.* Reference 4; L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgár and L. Pauling, THIS JOURNAL, **65**, 1940 (1943); A. Polgár and L. Zechmeister, *ibid.*, **66**, 186 (1944).

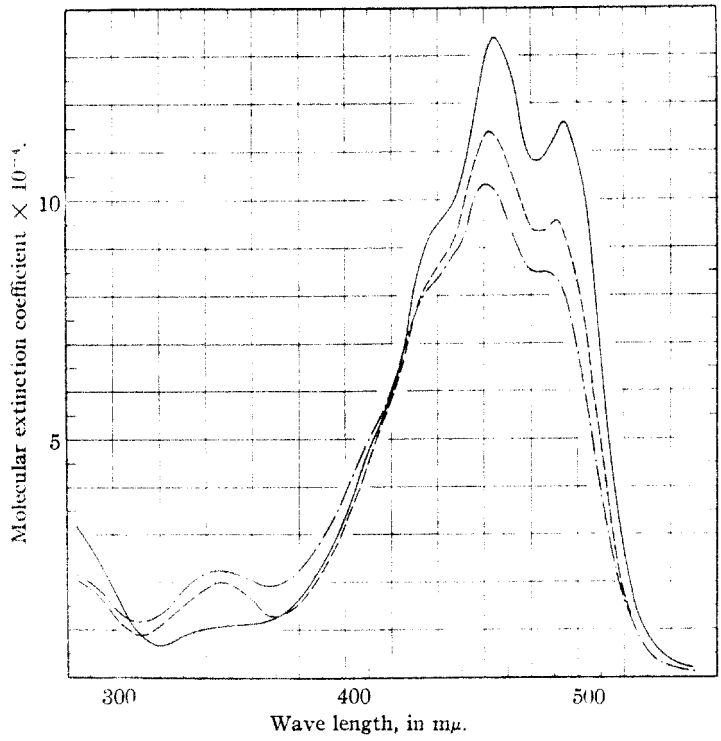


Fig. 1.—Molecular extinction curves of cryptoxanthin in benzene: ——, fresh solution of the all-*trans* compound; ---, mixture of stereoisomers after refluxing for forty-five minutes; — · — ·, after iodine catalysis at room temperature in light.

An insolation for forty-five minutes is adequate for cryptoxanthin; shorter exposures produce a slight stereoisomerization only. Zeaxanthin isomerizes to some extent in five minutes but an exposure of fifteen minutes was more satisfactory for the present stereochemical work. After thirty minutes of insolation most of the recovered pigment passed into the chromatographic filtrate under conditions under which any known member of the zeaxanthin set would have been adsorbed on the column.

The pigment mixture obtained upon insolation of zeaxanthin for fifteen minutes was composed of 48% unchanged all-*trans* compound, 11% neozeaxanthins, 12% minor zones, and 29% of a pigment which does not belong to the zeaxanthin set. The positions of the visual spectral bands of this latter pigment are approximately identical with that of the zeaxanthin bands. It is adsorbed between zeaxanthin and cryptoxanthin on the column. The investigation of this polyene, which was obtained in crystals in a yield of 17% from zeaxanthin, is not yet concluded.

We shall now consider the influence of *trans*→*cis* rearrangements on the spectral curves. As shown by Figs. 1 and 2, the molecular extinction curves of solutions which have been catalyzed with iodine resemble those of the carotenes in their essential features. There occurs upon catalysis a decrease of the extinction in the visible region which was observed earlier in heated solutions by Strain,[6] by Beadle and Zscheile,[7] and by White, Zscheile and Brunson[8]; partial spectral curves of some stereoisomers were given in those papers. We find that in the ultraviolet region a marked *cis*-peak appears at 348 mμ (in benzene). It must be stressed that light is required for *cis–trans* rearrangement of carotenoids induced by iodine[9]; neither cryptoxanthin nor zeaxanthin solutions showed a *cis*-peak when catalyzed in darkness. However, an irradiation with a lamp as short as five seconds developed a marked portion of the peak under conditions specified in the Experimental Part. The process is practically complete within two or three minutes.

The isolation of isomers from mixtures by chromatography permits the determination of the height of the *cis*-peak of each observed member of the set and, consequently, a tentative assignment of spatial structures.[4,5] The basis for the following discussion is given in Figs. 3, 4 and 5 as well as in Table II.

One *trans*→*cis* rotation usually shifts the longest wave length maximum of an all-*trans* carotenoid about 5 mμ toward the ultraviolet. Therefore, the neocryptoxanthins B and U are evidently mono-*cis*

TABLE II

TYPICAL SPECTROSCOPIC DATA FOR SOME MEMBERS OF THE STEREOISOMERIC CRYPTOXANTHIN AND ZEAXANTHIN SETS IN BENZENE SOLUTION (IN THE SEQUENCE OF DECREASING ADSORBABILITY)

Member of the set	Diff. between the visually established longest wave length max. of the member and the the all-*trans* form (mμ)	Mol. extinction coeff. at the *cis*-peak $E^{\text{mol.}}_{1\text{ cm.}} \times 10^{-4}$	Diff. between mol. ext. coeff of the member and of all-*trans* form at *cis*-peak
	Cryptoxanthin		
Neo U	5	1.7	0.7
All-*trans*	0	1.0	0
Neo A	6.5	3.4	2.4
Neo B	4.5	4.6	3.6
	Zeaxanthin		
Neo A	5.5	4.4	3.7
Neo B	5.5	2.4	1.7
Neo C	8.5	3.9	3.2
All-*trans*	0	0.7	0

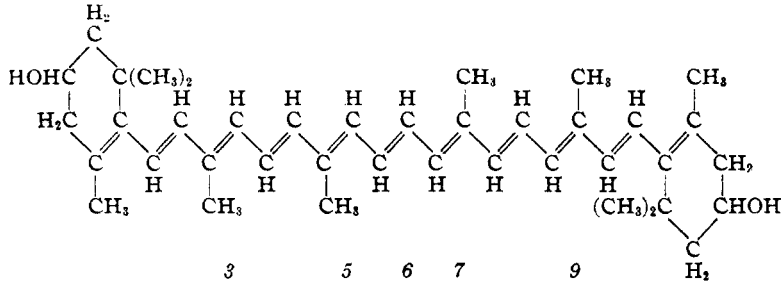
(6) H. H. Strain, ref. 2; *J. Biol. Chem.*, **127**, 191 (1938).

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

(8) 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).

(9) Cf. A. Polgár and L. Zechmeister, THIS JOURNAL, **66**, 186 (1944).

compounds. The very great difference in their *cis*-peaks suggests that the *cis* double bond is located in the center of the chromophore of neocryptoxanthin B but far from the center in that of neo U.[5] The most probable configurations are: neocryptoxanthin B = 6-mono-*cis*-cryptoxanthin, and neocryptoxanthin U = 3-mono-*cis*- or 9-mono-*cis*-cryptoxanthin (see the formula). Neocryptoxanthin A seems to be a di-*cis* compound in which the central double bond is one of the *cis* bonds.



All-*trans*-zeaxanthin (with one hydroxyl group: all-*trans*-cryptoxanthin). The stereochemically effective double bonds[10] are numbered.

In the stereoisomeric zeaxanthin set the neo forms A and B are undoubtedly mono-*cis* compounds. On the basis of their *cis*-peaks the best assignment is: neozeaxanthin A = 6-mono-*cis*-zeaxanthin and neozeaxanthin B = 5-mono-*cis*-zeaxanthin. Neozeaxanthin C seems to be a di-*cis* isomer; it is possible that one of its *cis* double bonds is located in the center.

Experimental Part

Materials and Methods.—Cryptoxanthin solutions were chromatographed on calcium hydroxide (Shell Brand lime, chemical hydrate, 98% through 325 mesh) and those of zeaxanthin on calcium carbonate (Merck Heavy Powder). The visual spectral maxima were determined with an Evaluating Grating Spectroscope (Zeiss; light filter BG-7; 2 mm. thick). All pigments listed in the chromatograms below were tested spectroscopically before and after the addition of catalytic amounts of iodine. The spectra of cryptoxanthin and its isomers were taken in petroleum ether (b. p. 60–70°) and those of zeaxanthin and its isomers in benzene. The addition of iodine shifted spectra in the former set to 480, 449 mμ (± 1 mμ) and in the latter to 493, 460 mμ (± 1 mμ). The extinction curves were taken in a Beckman photoelectric spectrophotometer.[11] Solutions which had been catalyzed with iodine were irradiated from a distance of 60 cm. with a 3500° Mazda white fluorescent lamp (length of the tubes, 120 cm.). The solutions were placed in 25-ml. volumetric flasks (glass) in horizontal position on a white base. For insolation experiments transparent quartz test-tubes or small flasks were used which were filled with carbon dioxide. For more details concerning methods we refer to earlier reports.[12]

The cryptoxanthin and zeaxanthin samples were isolated from the berries of *Physalis alkekengi* and, after purification, were chromatographically homogeneous.

1. Cryptoxanthin

(a) *cis–trans* Isomerization of Cryptoxanthin on Standing or Refluxing.—Petroleum ether solutions of cryptoxanthin isomerize at room temperature in darkness to an extent of 1% within twenty-four hours.

A solution of 6 mg. of the pigment in 50 ml. of ligroin (b. p. 120°) was refluxed for thirty minutes in an all-glass apparatus in a slow stream of carbon dioxide. The chromatogram was developed on a column (20 × 3.5 cm.) with petroleum ether containing 10% acetone. (The figures on the left are the width of the zones in millimeters).

5	yellow, irreversible
65	colorless
22	orange, all-*trans;* 483.5, 452.5 mμ
2	almost colorless
14	yellowish orange, neo A: 477, 445.5 mμ
2	colorless
12	yellow, neo B: 479.5, 448 mμ

The loss of the initial colorimetric value was about 30%.

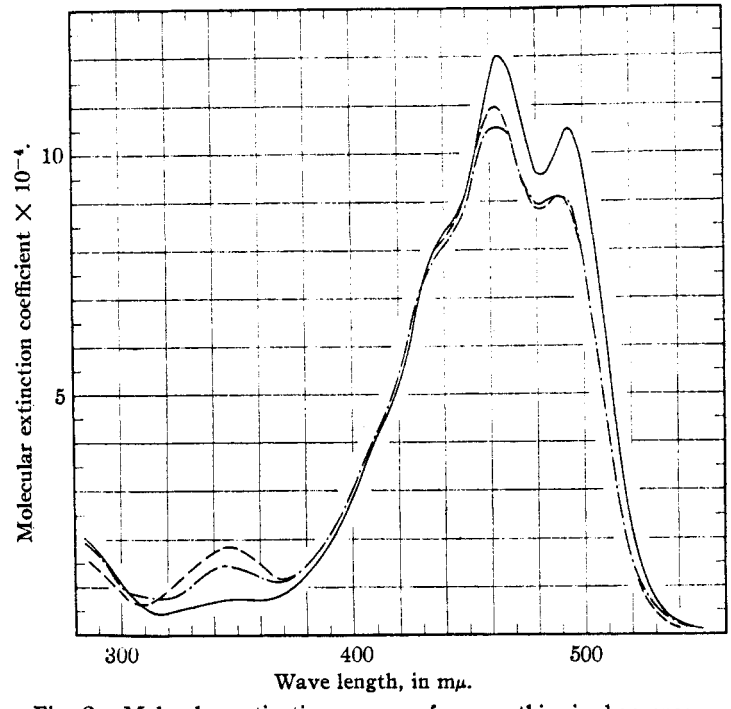


Fig. 2.—Molecular extinction curves of zeaxanthin in benzene: —, fresh solution of the all-*trans* compound; ---, mixture of stereoisomers after refluxing for forty-five minutes; and —·—·, after iodine catalysis at room temperature in light. (The *cis*-peak obtained on refluxing is higher than that of the iodine equilibrium because much more neozeaxanthin A is contained in the refluxed solution than in the catalyzed mixture[2].)

(10) 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).

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

(12) *Cf., e. g.*, A. Polgár and L. Zechmeister, THIS JOURNAL, **64**, 1856 (1942); L. Zechmeister and A. Polgár *ibid.*, **65**, 1522 (1943).

The colorimetric ratio was, unchanged all-*trans:* neo A: neo B = 62:32:6.

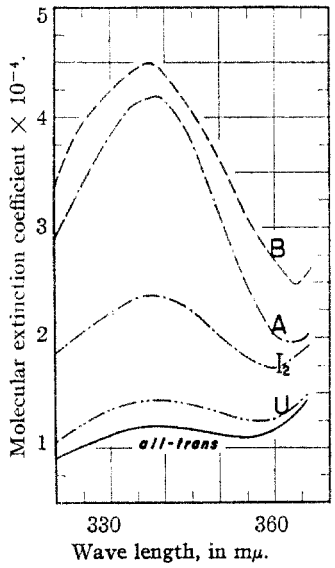


Fig. 3.—Molecular extinction curves of some members of the stereoisomeric cryptoxanthin set in the *cis*-peak region, in hexane. I_2 indicates the equilibrium mixture obtained upon iodine catalysis.

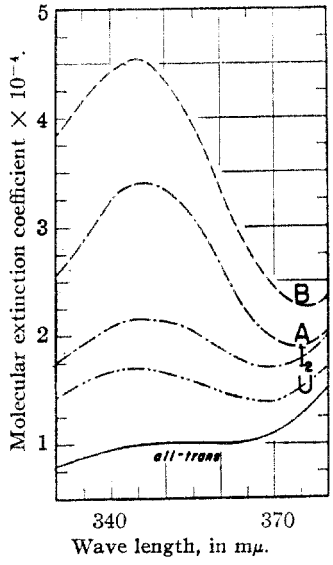


Fig. 4.—Molecular extinction curves of some members of the stereoisomeric cryptoxanthin set in the *cis*-peak region, in benzene. I_2 indicates the equilibrium mixture obtained upon iodine catalysis.

(b) *cis–trans* **Isomerization of Cryptoxanthin by Melting.**—Three milligrams of crystals was placed in a tube which was filled with carbon dioxide and then sealed. The tube was kept in a bath at 175° for fifteen minutes and then the melt was rapidly solidified in ice water. The petroleum ether solution of this material gave the following chromatogram (18 × 1.8 cm.) when the column was developed as in section (a).

1 brownish yellow, irrev.
75 colorless
15 yellowish orange, neo U: 478, 448 mμ
28 orange, all-*trans:* 483.5, 452 mμ
19 yellow, neo A: 477, 446 mμ
1 colorless
6 yellowish orange, neo B: 479, 448.5 mμ

The loss in the initial colorimetric value was 55%. The colorimetric ratio was, unchanged all-*trans:* neo U: neo A: neo B = 49:7:38:6.

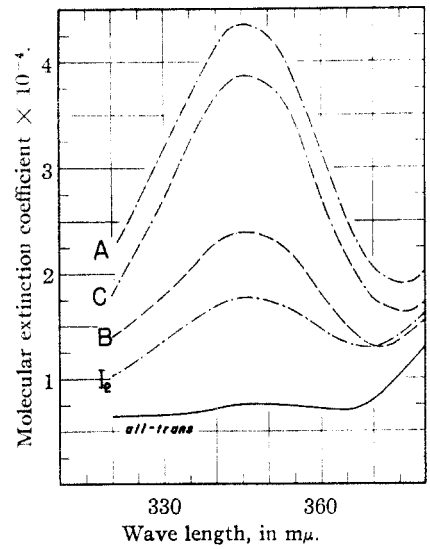


Fig. 5.—Molecular extinction curves of some members of the stereoisomeric zeaxanthin set in the *cis*-peak region, in benzene. I_2 indicates the equilibrium mixture obtained upon iodine catalysis.

(c) *cis–trans* **Isomerization of Cryptoxanthin by Iodine Catalysis at Room Temperature.**—A solution of 5 mg. of pigment in 30 ml. of petroleum ether was catalyzed with 0.1 mg. of iodine. An hour later the solution was chromatographed and, after development as above, gave the following zones (20 × 3.5 cm.).

2 brownish yellow, irrev.
110 colorless
12 yellowish orange, neo U: 478.5, 448 mμ
2 almost colorless
26 orange, all-*trans:* 483.5, 452.5 mμ
2 colorless
10 yellow, neo A: 477, 446 mμ
7 brownish yellow, neo B: 479.5, 449.5 mμ

Similar experiments were also carried out with the neo pigments. The relative colorimetric values of the zones thus formed are listed in Table III.

TABLE III

RELATIVE COLORIMETRIC VALUES OF CRYPTOXANTHIN AND SOME OF ITS STEREOISOMERS REVERSIBLY FORMED BY IODINE CATALYSIS AT 25° IN DIFFUSE DAYLIGHT

Starting material	Relative colorimetric values (% of the recovered pigment) neo U	all-*trans*	neo A	neo B
Neocryptoxanthin U	23	55	22	—
All-*trans*-cryptoxanthin	18	59	18	5
Neocryptoxanthin A	20	57	23	—
Neocryptoxanthin B	21	55	17	7

The influence of illumination on the iodine catalysis, especially on the *cis*-peak effect, is demonstrated in Table IV. In complete darkness no peak was observed one hour after the addition of iodine.

TABLE IV

INFLUENCE OF LIGHT ON THE DEVELOPMENT OF THE *cis*-PEAK IN IODINE-CATALYZED BENZENE SOLUTIONS OF CRYPTOXANTHIN AND ZEAXANTHIN

Duration of the illumination	Mol. extinction coeff. × 10^{-4} at the longest wave length max.	Mol. extinction coeff. × 10^{-4} at the *cis*-peak wave length	Increase in the *cis*-peak (% of the greatest change)
	Cryptoxanthin		
0 sec.	13.4	0.70	0
5 sec.	13.1	.81	10
30 sec.	12.7	1.16	42
2½ min.	12.0	1.62	84
15 min.	11.0	1.77	98
30 min.	10.0	1.79	100
	Zeaxanthin		
0 sec.	12.0	0.89	0
5 sec.	11.8	1.25	42
30 sec.	11.4	1.47	68
2½ min.	11.1	1.64	88
15 min.	10.8	1.74	100
30 min.	10.5	1.71	96
60 min.	10.1	1.74	100

(d) *cis–trans* **Isomerization of Cryptoxanthin by Insolation.**—A solution of 2 mg. of pigment in 30 ml. of petroleum ether was exposed for forty-five minutes to intense sunshine. The final temperature was 34°. The following chromatogram was obtained (18 × 1.8 cm.).

4 yellowish brown, irrev.
83 colorless
26 orange, all-*trans:* 483.5, 453 mμ
13 yellow, neo A: 478, 446.5 mμ

The loss of color was about 30% of the initial value. In the recovered pigment the colorimetric ratio was, all-*trans:* neo A = 86:14. In parallel experiments the presence of a small amount of neocryptoxanthin B was also noticed.

2. Zeaxanthin

(a) *cis–trans* **Isomerization of Zeaxanthin on Refluxing.**—The isomerization produced by refluxing benzene solutions has been described earlier.[2] When 5 mg. of zeaxanthin in 50 ml. of ligroin (b. p. 120°) was refluxed in darkness in an atmosphere of carbon dioxide, for thirty minutes, only small amounts of the usual main isomers, neo A and neo B, were formed whereas a considerable fraction was converted into pigments which do not belong to the zeaxanthin set. The following chromatogram was obtained on a carbonate column (20 × 3.5 cm.) after development with a mixture of equal volumes of petroleum ether and benzene.

8 pale yellow, irrev.
9 yellowish orange, neo A: 489.5, 457.5 mμ
5 almost colorless
6 yellowish orange, neo B: 490, 458 mμ
12 almost colorless
13 yellowish orange, irrev.: 489.5, 457.5 mμ
14 colorless
9 orange, all-*trans:* 494, 462.5 mμ
21 colorless
5 yellowish orange, irrev.: 491.5, 458.5 mμ

The loss in the initial color intensity was 57%. The colorimetric ratio was, all-*trans:* neo A: neo B = 73:14:13. About 35% of the total recovered pigment was distributed among the two irreversible zones whose spectra are listed. These pigments shifted their spectral bands upon addition of iodine in the expected manner but did not behave as dihydroxy compounds in the partition test.

(b) *cis–trans* **Isomerization of Zeaxanthin by Melting.**—A mixture of 5 mg. of crystals and 20 mg. of naphthalene was placed in a tube (filled with carbon dioxide). The sealed tube was kept in a bath at 160° for fifteen minutes and then rapidly cooled in ice water. The material was dissolved in cold benzene, diluted with petroleum ether, drawn into a column (20 × 3.5 cm.) and developed with a benzene–petroleum ether mixture (1:1).

22 almost colorless
6 yellowish orange, neo A: 489, 458 mμ
2 almost colorless
6 yellowish orange, neo B: 489, 457.5 mμ
6 colorless
4 yellowish orange, irrev.: 489.5, 457.5 mμ
5 colorless
5 yellow, neo C: 486, 454.5 mμ
9 colorless
13 orange, all-*trans:* 494.5, 462.5 mμ

The loss in color intensity was about 60%. Nearly ⅛ of the recovered pigment consisted of the irreversible zone which did not behave like a dihydroxy compound in the partition test. The colorimetric ratio was, all-*trans:* neo A: neo B: neo C = 56:17:17:10.

In another experiment, at 220° without naphthalene, almost complete bleaching occurred.

(c) *cis–trans* **Isomerization of Zeaxanthin by Iodine Catalysis at Room Temperature.**—The data published previously[2] have been confirmed. In addition, experiments showed no *cis*-peak in the presence of iodine within half an hour in darkness. The influence of irradiation on this process is illustrated in Table IV.

(d) *cis–trans* **Isomerization of Zeaxanthin by Insolation.**—A solution of 5 mg. of zeaxanthin in 50 ml. of benzene was exposed to intense sunshine for fifteen minutes. When this solution was developed with pure benzene on a column (20 × 3.5 cm.) the following zones resulted.

3 almost colorless
2 yellowish orange, neo A: 490.5, 459.5 mμ
2 colorless
2 yellowish orange, neo B: 490, 459 mμ
60 blurred, traces of pigment
10 orange, all-*trans:* 494, 462.5 mμ
13 colorless
4 yellowish orange, irrev.: 491.5, 458 mμ
8 yellowish orange, irrev.: 491.5, 458 mμ
26 colorless
2 yellowish orange, irrev.: 492.5, 460.5 mμ

The loss in the initial color intensity was 20%. The recovered pigment was composed of 48% unchanged all-*trans*-zeaxanthin, 11% neozeaxanthins A + B, 12% minor pigments, and 29% of the pigment contained in the 8 mm. zone.

The latter, irreversibly formed polyene was eluted with methanol, transferred into benzene and, after evaporation, crystallized from the minimum amount of benzene by the addition of excess methanol. When 100 mg. of zeaxanthin was insolated, the yield of the new recrystallized pigment was 17 mg. This pigment forms long plates which are tapered on both ends; m. p. 129–131° (cor., electrically heated Berl block; sealed tube filled with carbon dioxide). In the partition test the compound is intermediate between cryptoxanthin and the carotenes. The visually observed spectral maxima were: in petroleum ether, 482, 451 mμ; in benzene, 496.5, 463 mμ; in alcohol, 483.5, 452 mμ; and in chloroform, 495, 462 mμ. Except in alcohol solution, the addition of iodine caused a 2 to 4 mμ displacement of the maxima toward shorter wave lengths.

Summary

The *cis–trans* isomerization of cryptoxanthin and zeaxanthin has been investigated further. Earlier observations concerning neozeaxanthins were confirmed. Cryptoxanthin yielded two new stereoisomers, one of which is adsorbed above the all-*trans* compound on the Tswett column. In addition to previous methods, stereoisomerization also occurs upon the melting of

crystals or exposure of solutions to sunshine. Zeaxanthin is more photosensitive than cryptoxanthin. Extinction curves are given for fresh, refluxed, and iodine-catalyzed solutions. Cryptoxanthin and zeaxanthin develop *cis*-peaks at 348 mμ (in benzene); however, no peak was observed upon iodine catalysis in darkness. On the basis of optical data the most probable configurations are: neocryptoxanthin B = *6*-mono-*cis*-cryptoxanthin, and neocryptoxanthin U = *3*-mono-*cis*- or *9*-mono-*cis*-cryptoxanthin; neo A is a di-*cis* compound; neozeaxanthin A = *6*-mono-*cis*-zeaxanthin and neozeaxanthin B = *5*-mono-*cis*-zeaxanthin; neozeaxanthin C is probably a di-*cis* isomer.

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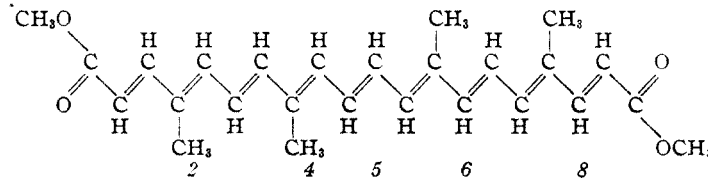
A Stereochemical Study of Methylbixin

BY L. ZECHMEISTER AND R. B. ESCUE

Bixin, $CH_3OOCC_{22}H_{26}COOH$, the main pigment in the seeds of the Annato tree (*Bixa orellana*, L.), was the first polyene for which stereoisomerism was demonstrated. Herzig and Faltis[1] in a single unreproducible experiment obtained from the seeds, instead of the well-known bixin, an isomer, termed "β-bixin," with higher melting point and greater stability than bixin, and with spectral maxima at longer wave lengths than those of bixin. Karrer and his associates[2] reported later that natural bixin can be converted into β-bixin by iodine, and they correctly interpreted this reaction as a *cis*→*trans* rearrangement. According to Kuhn and Winterstein[3] catalytic amounts of the halogen are sufficient to effect this transformation. Furthermore, they showed that both bixins yield the same dihydro compound, which is oxidized in air into β-bixin.

The following names have been used[4] for the two bixins and, in an analogous manner, for the two free carboxylic acids (norbixins) and the two dimethyl esters (methylbixins): ordinary bixin = natural bixin = *cis*-bixin = α-bixin = labile bixin = lower melting bixin = bixin II; isobixin = *trans*-bixin = β-bixin = stable bixin = higher melting bixin = bixin I.

Since no other isomer seems to have been described[5] and since the reversibility of the bixin isomerization by iodine catalysis so far as we know has not been claimed, we have re-investigated this field[6] by making use of some methods which were first applied to C_{40}-carotenoids.[7,8] Methylbixin was the most suitable starting material because of its greater solubility and markedly weaker adsorbability than that of bixin. Furthermore, because of its symmetrical molecule (see the formula) methylbixin can exist in only twenty stereoisomeric forms, whereas the corresponding number for bixin is thirty-two.[9,10]



All-*trans*-methylbixin

(The stereochemically effective double bonds are numbered.)

In the course of our experiments five members of the methylbixin set (and traces of other isomers) have been observed in chromatograms[11]; two new pigments, the neomethylbixins A and C, have been isolated as crystals. We designate

TABLE I

VISUALLY OBSERVED SPECTRAL MAXIMA OF SOME STEREOISOMERIC METHYLBIXINS LISTED IN THE SEQUENCE OF DECREASING ADSORPTION AFFINITIES

	In petroleum ether (b. p. 60–70°), mμ		In benzene, mμ	
Natural methylbixin	485	453.5	503	470
All-*trans*-methylbixin	490	457	508.5	475
Neomethylbixin A	485	454	502.5	469
Neomethylbixin B	471	444.5	491	458
Neomethylbixin C	479.5	449	496	463

(1) J. Herzig and F. Faltis, *Ann.*, **431**, 40 (1923).

(2) P. Karrer, A. Helfenstein, R. Widmer and Th. B. van Itallie, *Helv. Chim. Acta*, **12**, 741 (1929).

(3) R. Kuhn and A. Winterstein, *Ber.*, **65**, 646 (1932), and **66**, 209 (1933).

(4) *Cf.* L. Zechmeister, "Carotinoide," J. Springer, Berlin, 1934, pp. 239–251.

(5) A third bixin termed "isobixin" could not be reproduced; *cf.* J. F. B. van Hasselt, *Rec. trav. chim. Pays-bas*, **30**, 1 (1911), and **33**, 192 (1914); P. Karrer and T. Takahashi, *Helv. Chim. Acta*, **16**, 287 (1933).

(6) L. Zechmeister and R. B. Escue, *Science*, **96**, 229 (1942).

(7) *Cf. e. g.*, A. Polgár and L. Zechmeister, THIS JOURNAL, **64**, 1856 (1942); L. Zechmeister and A. Polgár, *ibid.*, **66**, 137 (1944).

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

(9) L. Pauling, *Fortschritte Chem. organ. Naturstoffe*, **3**, 203 (1939).

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

(11) A. Winterstein mentioned in Klein's "Handbuch der Pflanzenanalyse," Vol. IV, p. 1403 (1933), that *cis*- and *trans*-bixin can be separated chromatographically; no experimental directions were given; *cf.* A. Winterstein and R. Stein, *Z. physiol. Chem.*, **220**, 247 (1933).