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On Some Stereoisomeric Cryptoxanthins

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Although a first investigation of some stereoisomeric cryptoxanthins, $C_{40}H_{56}OH$, was reported earlier,² a more detailed study has now been made, considering the recently observed dependence of provitamin A activities on the shape of the molecule.³ Earlier, in collaboration with R. M. Lemmon,² the neocryptoxanthins U, A and B were obtained in solution only and a few spectroscopic data were made available. In the present paper we wish to report on the isolation of the neocryptoxanthins U and A in the form of analytically pure crystals, and on the further purification and improved characterization of solutions of neocryptoxanthin B.

The chromatographic sequence of the pure products was, neo-U (top), all-*trans*, neo-A and neo-B. The full extinction curves of the three *cis* isomers are given in Figs. 1-3, and some characteristic spectroscopic data are compiled in Table I; a few data² referring to neo-B had to be revised.

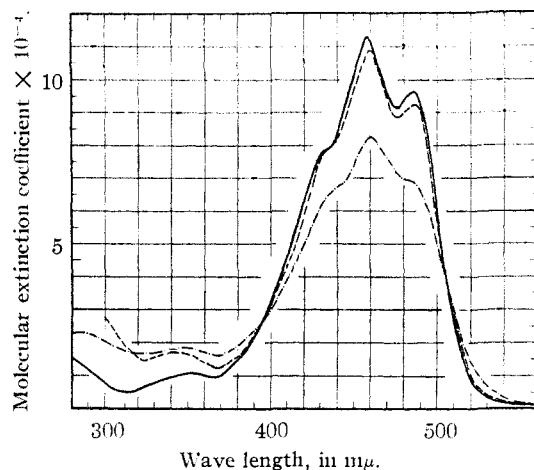


Fig. 1.—Molecular extinction curve of neocryptoxanthin U in benzene: —, fresh solution of neocryptoxanthin U; — — —, mixture of stereoisomers after refluxing for forty-five minutes in darkness; — · — ·, after iodine catalysis at room temperature in light (in this particular case considerable photochemical destruction depresses E_{max} of the iodine catalyzed mixture).

* Editorial Board 1948—

- (1) Née Mookerjee (Fellow of the Indian Government).
- (2) L. Zechmeister and R. M. Lemmon, *THIS JOURNAL*, **66**, 317 (1944); cf. L. Zechmeister and P. Tuzson, *Biochem. J.*, **32**, 1305 (1938) and *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) H. J. Deuel, Jr., E. R. Meserve, A. Sandoval and L. Zechmeister, *Arch. Biochem.*, **10**, 491 (1946); H. J. Deuel, Jr., S. M. Greenberg, E. Straub, T. Fukui, A. Chatterjee and L. Zechmeister, *Arch. Biochem.*, **23**, 239 (1949). Further literature: L. Zechmeister, *Vitamins and Hormones*, **7**, 57 (1949).

Neocryptoxanthin U, the only known member of this stereoisomeric set which has stronger adsorption affinity than the all-*trans* form and shows a rather flat *cis*-peak section in its extinction curve, should be classified (as before) as a mono-*cis*-cryptoxanthin with a peripherally located *cis* double bond. It belongs to the same type as neo- α -carotene U or neo- β -carotene U.

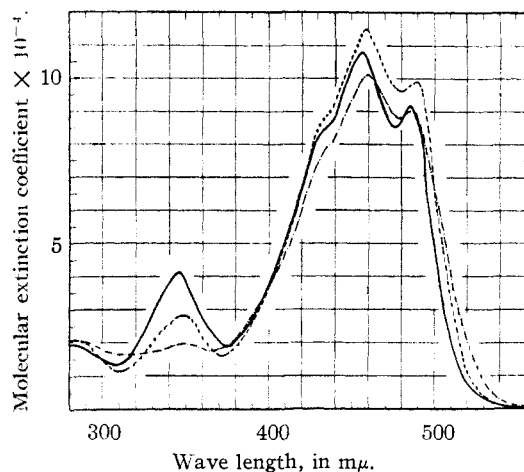


Fig. 2.—Molecular extinction curve of neocryptoxanthin A in benzene: —, fresh solution of neocryptoxanthin A; — — —, mixture of stereoisomers after refluxing for forty-five minutes in darkness; — · — ·, after iodine catalysis at room temperature, in light.

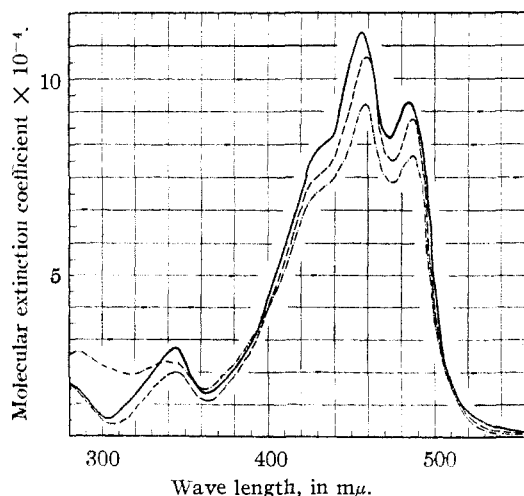


Fig. 3.—Molecular extinction curve of neocryptoxanthin B in benzene: —, fresh solution of neocryptoxanthin B; — — —, mixture of stereoisomers after refluxing for forty-five minutes in darkness; — · — ·, after iodine catalysis at room temperature, in light.

We believe that the other main product of the cryptoxanthin stereoisomerization, *viz.*, neo-A, which shows a pronounced *cis*-peak, contains a centrally located *cis* double bond in its molecule. However, it cannot be decided with reliability, whether or not a second such bond is present in peripheral position.

As described in the Experimental Part, it is easy to obtain neocryptoxanthin U in crystalline form (Fig. 4). However, following a similar procedure for the isolation of the neocryptoxanthin A, we first obtained crystallizates whose freshly prepared solutions contained one-third of the pigment in the all-*trans* configuration as demonstrated by a subsequent chromatogram. If the temperature is kept below 30° during the total isolation procedure, chromatographically homogeneous neocryptoxanthin A crystals can be obtained (Fig. 5). This homogeneity disappeared, however, and a mixture was formed containing 30% all-*trans* isomer when pure neo-A crystals were kept at 56°, in high vacuum, for a few hours. No steric change took place in a parallel experiment carried out with neocryptoxanthin U crystals.

TABLE I

POSITION OF THE MAIN MAXIMA OF SOME STEREOISOMERIC CRYPTOXANTHINS IN THE VISIBLE SPECTRAL REGION

	Determined photo-electrically (Beckman spectrophotometer)		Observed visually (Zeiss grating spectroscope; light filter, Jena BG7)			
	In benzene, m μ		In benzene, m μ		In petroleum ether (b. p. 60-70°), m μ	
All- <i>trans</i> -cryptoxanthin	492	464	497	463	485	452.5
Neocryptoxanthin U	486	458	493	458.5	481	448.5
Neocryptoxanthin A	484-485	456	490.5	456.5	478	446.5
Neocryptoxanthin B	484	456	490.5	457.5	478	447.5

An analogous influence of the position of the *cis* double bond on the thermostability was observed when freshly prepared benzene solutions of crystals were refluxed in darkness, in nitrogen atmosphere, for forty-five minutes. When chromatographically homogeneous neocryptoxanthin U was treated in this manner, a subsequent resolution on the Tswett column gave the following ratios for the final mixture. Unchanged neo-U: all-*trans*-form (including a little neo-A) = 90:10. In contrast, when neo-A was used as a starting material, the ratio was, unchanged neo A: all-*trans*-form (including a little neo U) = 42:58. No such differences appeared when benzene solutions were kept at 4°. Under these conditions neocryptoxanthin A remained chromatographically and hence sterically homogeneous for several days; neither could any spectroscopic change be observed.

It was reported earlier that all-*trans*-cryptoxanthin solutions suffered marked stereoisomerization when exposed to sunlight. The same phenomenon takes place, during a shorter illumination period, under the influence of the usual light

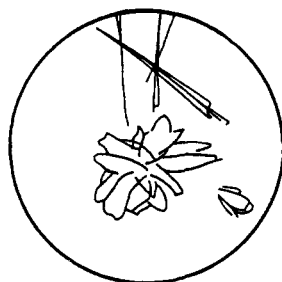


Fig. 4.—Neocryptoxanthin U, crystallized from benzene and methanol (about 200 ×).

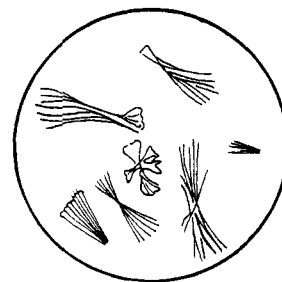


Fig. 5.—Neocryptoxanthin A, crystallized from benzene and methanol (about 200 ×).

source ("Punktlichtlampe") of the Zeiss Grating Spectroscope. For this reason readings should be made rapidly. After a fifteen-minute illumination of a benzene solution, in a glass cell, from 43 cm. distance, the all-*trans*-form remained unchanged, while in parallel experiments 20% of the neo-U and 10% of the neo-A form was converted into all-*trans*-cryptoxanthin.

TABLE II

MOLECULAR EXTINCTION COEFFICIENTS OF SOME STEREOISOMERIC CRYPTOXANTHINS IN BENZENE (THE WAVELENGTHS OF THE MAXIMA ARE ITALICIZED)

Neocryptoxanthin U	Neocryptoxanthin A		Neocryptoxanthin B	
	$E_{1\text{ cm.}}^{\text{mol.}} \times 10^{-4}$	m μ	$E_{1\text{ cm.}}^{\text{mol.}} \times 10^{-4}$	m μ
300	0.76	300	1.45	300
400	3.25	<i>346</i>	4.07	<i>345</i>
440	8.40	360	2.98	360
<i>458</i>	11.3	400	3.86	400
470	9.56	<i>456</i>	10.8	<i>456</i>
476	9.16	470	9.02	470
<i>486</i>	9.67	475	8.57	475
490	9.24	<i>484-485</i>	9.05	<i>484</i>
		490	8.70	490

Experimental

Isolation of Cryptoxanthin.—The procedure described earlier⁴ was improved as follows: 45 kg. of persimmons were broken up by hand and ground in Waring Blenders with a total of 45 l. of methanol. The next day the dehydrated pulp was centrifuged in a basket centrifuge; the pulp (divided into 10 portions) was suspended in a (total) mixture of 45 l. of petroleum ether (b. p. 60-70°) and 15 l. of methanol. After mechanical shaking for one-half hour, subsequent centrifuging and washing in the centrifuge with 20 l. of petroleum ether-methanol (1:1) the extraction was practically complete. The pigment was then transferred into petroleum ether by the addition of water; the solution was washed free of methanol, dried with sodium sulfate, concentrated *in vacuo* to 4 l. and saponified over 20% methanolic potassium hydroxide overnight. After washing, drying and concentrating to 1 l., the extract was developed on calcium hydroxide ("Sierra Hydrated Lime")-celite 545 (3:1) in a conical percolator (45 × 20 × 10 cm.) with petroleum ether containing 6% acetone. The 6-cm. broad main zone was eluted with methanol, transferred into petroleum ether, dried, concentrated to 1 l., rechromatographed using four cylindrical columns (30 × 8 cm., petroleum ether-8% acetone). The

(4) H. J. Deuel, Jr., E. R. Meserve, A. Sandoval and L. Zechmeister, *Arch. Biochem.*, **10**, 491 (1948).

combined extracts from the main zone were evaporated to dryness and the cryptoxanthin was crystallized from benzene + methanol; yield 426 mg. After three recrystallizations, 300 mg. of analytically pure crystals were obtained: $E_{1\text{ cm.}}^{\text{mol.}} = 14.0 \times 10^4$ at 451 $m\mu$ (in hexane); $E_{1\text{ cm.}}^{\text{mol.}} = 13.55 \times 10^4$ at 464 $m\mu$ (in benzene).

Neocryptoxanthin U.—Thirty-six portions, each of 6 mg. of cryptoxanthin in 30 ml. of petroleum ether, were illuminated (in 50-ml. volumetric flasks) in the presence of 0.1 mg. of iodine with two 3500° Mazda lamps (length of tube, 120 cm.; distance, 60 cm.) for forty-five minutes. The combined solution was developed on eighteen calcium hydroxide-celite columns (26 × 5.8 cm.) with petroleum ether–8% acetone, for three hours. The main section of each chromatogram was composed of an intensely orange all-*trans* zone, above which the dull brownish-orange neo-U zone was located, while under the all-*trans* zone several yellow zones appeared (the latter constituted the starting material for the isolation of neocryptoxanthin A). The eighteen combined neo-U zones were rechromatographed on thirty-six columns. Although only a 2–3 mm. colorless interzone appeared between neo-U and marked amounts of the all-*trans* form, a clean isolation of the former was possible when the column was cut. In order to increase yield, the iodine catalysis was repeated twice by using the combined zones of unchanged all-*trans*-cryptoxanthin; finally, all neo-U fractions were combined, eluted with methanol, transferred into petroleum ether, washed fourteen times with doubly distilled water (in order to eliminate ash), completely evaporated, dissolved in a minimum amount of benzene and crystallized in a centrifuge tube by cautious addition of methanol at room temperature. After standing overnight in the cold room, the crystals were collected by centrifugation and were washed repeatedly with ice-cold methanol, the yield was 20 mg. For analysis the sample was recrystallized and dried in high vacuum at 56°; m. p. 95–96° (cor., Berl block, sealed capillary tube).

Anal. Calcd. for $C_{40}H_{56}O$: C, 86.87; H, 10.22. Found (after correction for 0.5% ash): C, 86.56; H, 10.66.

Neocryptoxanthin U forms long needles or, when slowly crystallized, characteristic plates (Fig. 4). It is consider-

ably more soluble in benzene or methanol than its all-*trans* isomer.

Neocryptoxanthin A.—The yellow zones mentioned, located under the all-*trans* form, were rechromatographed on three calcium hydroxide columns (26 × 5.8 cm.) and developed with 6% acetone-ligroin. The combined main zones constituted the starting material for the isolation of neo-A and the minor zone immediately below them that for neo-B. The neo-A zones appeared, after rechromatography, to be homogeneous. They were eluted with methanol, transferred into petroleum ether, washed, dried and evaporated to dryness *in vacuo* without delay at 22–24°. The oily residue was taken up in two drops of benzene and crystallized in a small centrifuge tube by cautious addition of methanol with stirring at room temperature (Fig. 5); yield, after washing with ice-cold methanol and recrystallization; 5 mg., m. p. 75–76° (cor.), $E_{1\text{ cm.}}^{\text{mol.}}$ at $\lambda_{\text{max.}} = 10.4 \times 10^4$ (established by weighing) and 10.8×10^4 (indirect determination based on iodine "equilibrium" data). For analysis the sample was dried in high vacuum at room temperature for three hours.

Anal. Calcd. for $C_{40}H_{56}O$: C, 86.87; H, 10.22. Found (after correction for 0.55% ash): C, 87.68; H, 10.62.

Neocryptoxanthin A is more soluble in benzene, petroleum ether or methanol than neo-U or the all-*trans* form.

Neocryptoxanthin B.—The total amount of this isomer (see above) was about 2 mg. This isomer is best chromatographed by using petroleum ether + 5% acetone on a calcium hydroxide-celite column. Although neo-B has not been crystallized, quantitative extinction curves are given in Fig. 3 on the basis of concentrations as established by iodine catalysis, as we believe with an accuracy of $\approx 5\%$.

Summary

Three *cis-trans* isomers of cryptoxanthin $C_{40}H_{56}OH$, are described and their relative stabilities as well as spectral characteristics are discussed.

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2,5-Dimethylcyclopentanecarboxylic Acids

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The stereoisomeric 2,5-dimethylcyclopentanecarboxylic and 2,6-dimethylcyclohexanecarboxylic acids offer an opportunity for the study of steric influences on the relative reactivity of a series of closely related compounds. A comparison of the corresponding cyclopentane and cyclohexane stereoisomers is of special interest because differences should arise largely from the non-planar nature of the latter. This paper describes the synthesis of the cyclopentane compounds and qualitative experiments on their reactivity.

2,5-Dimethylcyclopentanecarboxylic acid exists in four stereoisomeric forms. Two of these, Ia and Ib, are enantiomers while II and III are meso

forms. The racemic modification and one pure meso form were prepared by Wislicenus and co-workers² who also isolated in very poor yield a low-melting acid which we have shown to be an eutectic mixture of the two meso forms. Their synthesis was used in the present work with modifications which permitted isolation of the second meso form. This synthesis is represented by the equations shown.

The diacid m. p. 201–202° (decarb.) was assigned configuration V by Wislicenus² because it gave a single monocarboxylic acid, Iab, m. p. 49–50° on heating, whereas IX, m. p. 190–194° (decarb.), gave a mixture of II and III. We have confirmed this configuration by partial resolution of Iab and by preparing from IX a second acid

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(1) An abstract of part of a thesis submitted by Warner H. Florsheim in partial fulfillment of the requirements for the Ph.D. degree in chemistry, May, 1948. A preliminary report of this work was presented before the Organic Division at the 112th meeting of the American Chemical Society, New York City, September, 1947.

(2) (a) Wislicenus, Peters, Schramm and Mohr, *Ber.*, **34**, 2565 (1901); (b) Peters, Inaugural Dissertation, Leipzig, 1892; (c) Schramm, Inaugural Dissertation, Leipzig, 1895; (d) Mohr, Inaugural Dissertation, Leipzig, 1901.