[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 1815]

Action of N-Bromosuccinimide on β-Carotene

BY L. ZECHMEISTER AND L. WALLCAVE

RECEIVED MAY 12, 1953

β-Carotene when refluxed with N-bromosuccinimide yields a complicated pigment mixture from which three compounds have been isolated in pure state, *viz.*, dehydro-β-carotene, $C_{40}H_{54}$, bisdehydro-β-carotene, $C_{40}H_{52}$, and a pigment very probably identical with anhydro-eschscholtzxanthin, $C_{40}H_{50}$. Some spectral and stereochemical observations are discussed.

So far as we know direct dehydrogenations of polyene hydrocarbons belonging to the carotenoid series have been carried out only in a few instances and have started from aliphatic structures. Karrer and Rutschmann[1] have shown eight years ago that the tomato pigment, lycopene, $C_{40}H_{56}$, can be converted into dehydrolycopene, $C_{40}H_{52}$, and recently it was reported from our laboratory, in collaboration with B. K. Koe,[2] that two colorless, fluorescent members of this series, phytofluene and phytoene, can be converted by treatment with N-bromosuccinimide into carotenoid pigments containing, respectively, 7, 9 and 11 conjugated double bonds.

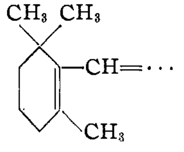
We observed that a carbon tetrachloride solution of β-carotene (which contains two terminal ring systems) shows considerable deepening of the color when refluxed with N-bromosuccinimide. Under the conditions applied a complicated pigment mixture was formed which contained about 25% unchanged starting material, while 15% consisted of identified dehydrogenation products of β-carotene. This latter fraction was resolved chromatographically to yield the following three main compounds that were isolated in analytically pure, crystalline form.

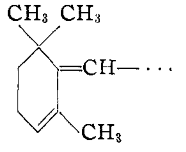
1. Dehydro-β carotene,[3] which thus has been obtained by direct dehydrogenation for the first time.
2. A new polyene more deeply colored than the former (bisdehydro-β-carotene).
3. The third product showed the properties of anhydro-eschscholtzxanthin, a pigment prepared by Karrer and Leumann[4] by elimination of water from Strain's eschscholtzxanthin.[5] Although a direct comparison with an authentic sample could not be carried out, we tentatively identify our substance to be anhydro-eschscholtzxanthin.

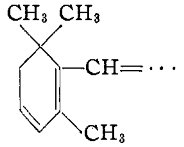
The lengths of the conjugated systems in the dehydrogenation products mentioned are indicated by the location of their spectral maxima (observed visually in hexane):

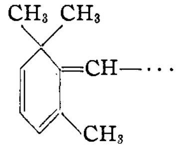
Anhydro-eschscholtzxanthin,	$C_{40}H_{50}$:	534, 499 mμ
Bisdehydro-β-carotene,	$C_{40}H_{52}$:	522, 490
Dehydro-β-carotene,	$C_{40}H_{54}$:	504, 474
β-Carotene,	$C_{40}H_{56}$:	484, 454

The characteristic end groups of these compounds can be formulated as follows (⌈ = double bond):


β-Carotene
(11 ⌈)


Dehydro-β-carotene
(12 ⌈)


Bisdehydro-β-carotene(?)
(13 ⌈)


Anhydro-eschscholtzxanthin
(14 ⌈)

The chromatographic sequence of these dehydrogenation products is rather surprising since it is not determined by the number of conjugated double bonds alone. While β-carotene (11 conj. ⌈) is adsorbed above α-carotene (10 conj. ⌈), the top-to-bottom sequence of the dehydrogenation products is, dehydro-β-carotene, bisdehydro-β-carotene and anhydro-eschscholtzxanthin. The intermediate position of the bisdehydro compound with reference to adsorption affinity, number of double bonds, and position of the spectral maxima, would also permit a non-symmetrical placement of the chromophore in the C_{40}-skeleton; in that case, one end group of the bisdehydro-β-carotene molecule would have to be formulated as that of dehydro-β-carotene while the other one would structurally coincide with the cyclic groups of anhydro-eschscholtzxanthin.

Bisdehydro-β-carotene is subject to *trans* → *cis* isomerization when catalyzed with iodine in light. Three main *cis* forms then appear which all have weaker adsorption affinities than the all-*trans* compound. It is characteristic of this set that in such quasi-equilibrium mixtures about two thirds of the pigment consists of *cis* compounds *versus* one third *trans*. This ratio corresponds roughly to that observed upon similar treatment of dehydro-β-carotene but is quite different from corresponding mixtures, obtained by stereoisomerization of naturally occurring carotenes, in which the *trans* form is consistently preponderant. As indicated in Figs. 1 and 2, the spectral curves of the *cis* bisdehydro-β-carotenes are surprisingly similar to that of the all-*trans* form and they do not show *cis*-peaks. When ordinary (*i.e.*, non-"retro")[6] carotenoids undergo *trans* → *cis* changes, a *cis*-peak appears at wave lengths (in hexane) about 142 mμ

---

[1] P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, **28**, 793 (1945).

[2] B. K. Koe and L. Zechmeister, *Arch. Biochem. Biophys.*, **41**, 236 (1952); dehydrogenation of squalene: J. Dale, *ibid.*, p. 475.

[3] R. Kuhn and E. Lederer, *Ber.*, **65**, 637 (1932); P. Karrer and G. Schwab, *Helv. Chim. Acta*, **23**, 578 (1940).

[4] P. Karrer and E. Leumann, *ibid.*, **34**, 445 (1951).

[5] H. H. Strain, *J. Biol. Chem.*, **123**, 425 (1938).

[6] *Cf.* W. Oroshnik, G. Karmas and A. D. Mebane, THIS JOURNAL, **74**, 295 (1952).

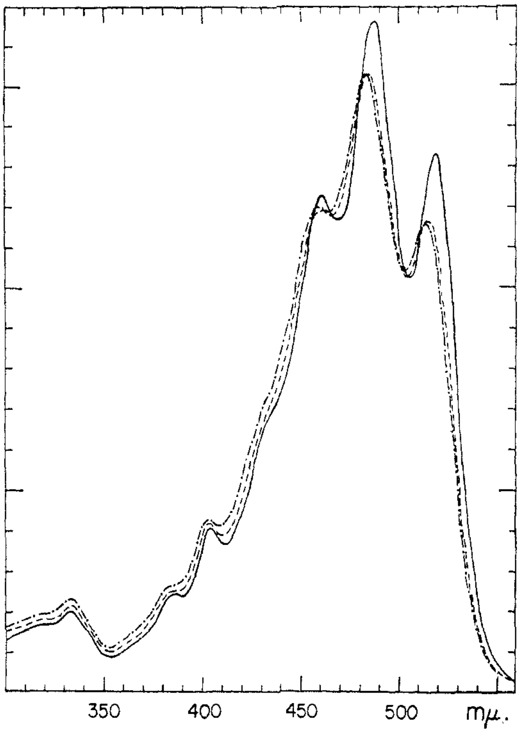


Fig. 1.—Molecular extinction curve in hexane of all-*trans*-bisdehydro-β-carotene: ——, fresh solution; – – – –, after refluxing for 1 hour; –·–·–, after iodine catalysis, in light.

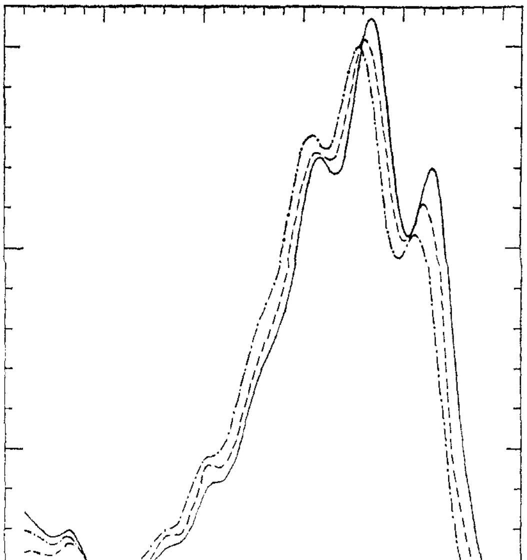


Fig. 2.—Molecular extinction curves in hexane of some *cis*-bisdehydro-β-carotenes: —, neo A; – – – –, neo B; –·–·–, neo C.

shorter than the position of the longest wave length maximum. Hence, the expected position of a *cis*-peak in the bisdehydro-β-carotene set lies around 375 mμ. Although no such peak appears in that region, the minor maxima located at 405 and 385 mμ in the all-*trans* curve increase markedly upon stereoisomerization while the extinction values in the main band decrease (Figs. 1–2). It is also noteworthy that the wave length difference between λ_{max} and the first overtone amounts only to 150 mμ, *versus* 175 mμ in the β-carotene set. The same statement is valid for the "retro" compounds, dehydro-β-carotene and anhydro-eschscholtzxanthin (Fig. 3).

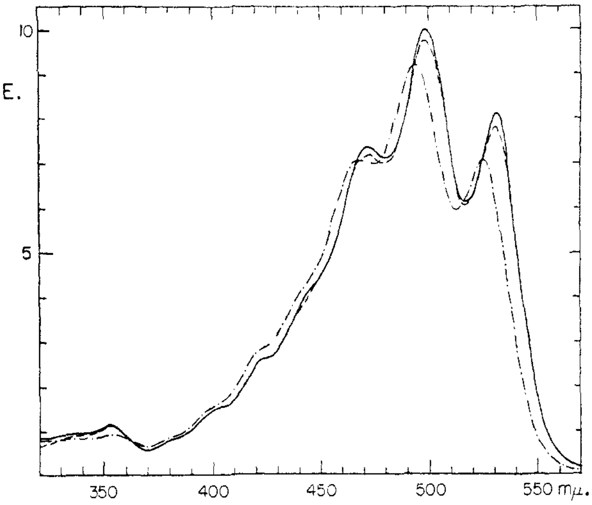


Fig. 3.—Extinction curves in hexane of anhydro-eschscholtzxanthin: —, all-*trans*; – – –, neo A; –·–·–, neo B.

According to Drs. H. J. Deuel, Jr., and J. Ganguly of the University of Southern California, bisdehydro-β-carotene does not act as a provitamin A in the rat.

Experimental

Materials and Methods.—The N-bromosuccinimide used was manufactured by Arapahoe Chemicals, Inc., Boulder, Colorado. The lime–celite was a 2:1 mixture of Sierra Hydrated Lime, Superfine (U.S. Lime Products Corp., Los Angeles, Calif.) and Celite No. 545 (Johns–Manville Co.). Elutions were carried out with acetone, the pigment was transferred by the addition of water to hexane and washed in a continuous apparatus. Melting points (cor.) were taken in an electrically heated Berl block, in evacuated capillaries. Visually observed spectra refer to a Zeiss Evaluating Grating Spectroscope (Loewe–Schumm), while the extinction curves were taken in the Beckman instrument.

Reaction of β-Carotene with N-Bromosuccinimide.—To a solution of 200 mg. of β-carotene in 60 ml. of carbon tetrachloride, contained in a 100-ml. round-bottom flask, 130 mg. of the reagent was added (molecular ratio, 1:2), and the liquid refluxed for 6 hours. The cooled solution, then markedly darker than initially, was filtered from succinimide crystals, concentrated *in vacuo* to 20 ml., diluted with 80 ml. of hexane, adsorbed on a 27 × 8 cm. column and developed with hexane containing 3% acetone (figures on left represent thickness of zones, in mm.):

15 brownish-red
10 interzone
75 several red and orange zones
40 interzone
45 yellow-orange: unchanged β-carotene (50 mg.)

The recovered β-carotene was rechromatographed, crystallized and identified by spectral and mixed chromatographic tests and was made use of in further experiments.

The eluate of the 75-mm. section was transferred into hexane and evaporated completely. The crystalline pigment mixture was then transferred into a small centrifuge tube, using the minimum necessary amount of warm benzene, and crystallized at 50° by the dropwise addition of methanol, with stirring. The centrifuged crystals (28 mg.)

were dissolved in 150 ml. of hexane at 20° and developed on a 27 × 8 cm. column with a 1:3 mixture of benzene–hexane (the following spectral maxima, in mμ, were observed visually, in hexane solutions):

48 minor zones and interzones
25 orange-red: all-*trans*-dehydro-β-carotene (504, 474)
50 reddish-purple: all-*trans*-bisdehydro β-carotene (522, 490)
25 purple: anhydro-eschscholtzxanthin (534, 499)
15 orange: neo-dehydro-β-carotene A (500, 469)
10 orange-red: a *cis*-bisdehydro-β-carotene (518, 489)
5 pale orange: neo-dehydro-β-carotene D (494, 461)
3 interzone
10 pale orange: a *cis*-bisdehydro-β-carotene (515, 484)
5 yellow-orange: a *cis*-dehydro-β-carotene (491, 460)
5 interzone
10 pale orange: a *cis*-bisdehydro-β-carotene (510, 481)

The identity of the stereochemical sets to which the respective *cis* forms belonged was established by iodine catalysis and mixed chromatography of the all-*trans* compound thus formed with an authentic sample of the corresponding substance. Each zone was eluted and transferred into benzene–hexane.

Dehydro-β-carotene.—The dried benzene–hexane solution was evaporated and the crystalline residue recrystallized from benzene–methanol as described above for the crude pigment mixture; yield 3–4 mg., m.p. 192°. This compound was identified by spectroscopic readings, mixed chromatogram with a sample *ex* β-carotene iodide, and by its behavior in isomerization experiments.

Anal. Calcd. for $C_{40}H_{54}$: C, 89.83; H, 10.17. Found: C, 89.79; H, 9.91.

Bisdehydro-β-carotene.—This fraction was evaporated, the residue dissolved in benzene and crystallized by cautious addition of methanol at 50°; yield 2–2.5 mg., m.p. 204°. From various experiments about 35 mg. of this compound was isolated as long quadrangular plates with slightly curved endings. Macroscopically the crystals appear to be definitely more purple than those of dehydro-β-carotene. Under the microscope single crystals are pale purple and crossings may appear very dark, almost black. Sparingly soluble in hexane, better in benzene, insoluble in methanol. When partitioned between hexane and 95% methanol, the pigment is found entirely in the epiphase. For the position on the lime–celite columns, *cf.*, the chromatogram above. Molecular extinction coefficient at $\lambda_{\max}$ in hexane: $E^{\text{mol.}}_{1\text{ cm.}}$ 16.6×10^4 at 487 mμ.

Anal. Calcd. for $C_{40}H_{52}$: C, 90.16; H, 9.84. Found: C, 89.40; H, 10.37.

Catalytic hydrogenation: 5.269 mg. substance in cyclohexane–glacial acetic acid (1:1) added, in the presence of 11.5 mg. of PtO_2, 2.76 ml. of hydrogen (0°, 760 mm.); calcd. 13 double bonds, found 12.5 double bonds. A parallel experiment carried out with β-carotene gave the following result: calcd. 11 and found 10.6 double bonds.

When bisdehydro-β-carotene is catalyzed with iodine in light, the three main *cis* forms (neos A-C) can be separated from the all-*trans* compound by developing with a 1:3 benzene–hexane mixture on lime–celite, whereby clear interzones are obtained. Visually observed maxima in hexane of the rechromatographed stereoisomers: all-*trans*-bisdehydro-β-carotene 521, 490, 457 mμ; neo A: 518, 485, 456 mμ; neo B: 514, 483 mμ; and neo C: 510, 481 mμ.

Anhydro-eschscholtzxanthin.—The benzene–hexane solution of this compound was evaporated and the pigment crystallized from benzene–methanol as described; yield 1 mg.; in all, 14 mg. was obtained from parallel experiments, dark purple crystals, m.p. 193°. Under the microscope bulky conglomerates appear but parallel twins and fan-like groupings are also found. Single crystals are purple with a brownish tinge while overlapping units are nearly black. The pigment is more soluble in hexane or benzene than bisdehydro-β-carotene; it is insoluble in methanol. When partitioned between hexane and 95% methanol, it shows entirely epiphasic behavior. It is adsorbed just below bisdehydro-β-carotene. The visually observed bands are located at 534, 499 mμ in hexane. Molecular extinction coefficient at $\lambda_{\max}$, in hexane: $E^{\text{mol.}}_{1\text{ cm.}}$ 16.0×10^4 at 499 mμ.

Anal. Calcd. for $C_{40}H_{50}$: C, 90.50; H, 9.50. Found: C, 89.89; H, 10.10.

The chromatogram of a solution that had been kept at 4° for 3 days showed two main *cis* forms, both adsorbed below the *trans* zone.

Acknowledgment.—We wish to thank Professor A. J. Haagen-Smit, Mr. G. Swinehart and Dr. A. Elek for analyses.

PASADENA, CALIF.

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 1813]

Conversion of Dehydro-β-carotene, *via* its Boron Trifluoride Complex, into an Isomer of Cryptoxanthin[1]

BY L. WALLCAVE AND L. ZECHMEISTER

RECEIVED APRIL 17, 1953

Dehydro-β-carotene and BF_3-etherate form a dark blue complex. Cleavage with water (or methanol) resulted in the isolation of a monohydroxy-β-carotene, termed isocryptoxanthin (or its methyl ether). Isocryptoxanthin when treated with acid chloroform undergoes dehydration to yield dehydro-β-carotene which indicates the presence of an allylic hydroxyl group. While naturally occurring cryptoxanthin is 3-hydroxy-β-carotene, isocryptoxanthin is the corresponding 4-compound. Its methyl ether or acetate can also be converted into dehydro-β-carotene.

Dehydro-β-carotene, $C_{40}H_{54}$, which so far as we know does not occur in nature, was obtained by Kuhn and Lederer[2] starting from β-carotene iodide. Its symmetrical structure was proposed by Karrer and Schwab[3] and is, in Oroshnik's terminology,[4] a "retro" structure (see the formula below), *i.e.*, the aliphatic section of the molecule and the rings are connected by double bonds.

A brief remark was made by Strain[5] concerning the formation of a dark blue coloration by the interaction of carotenoids and boron trifluoride. Somewhat earlier Lewis and Seaborg[6] reported on similar phenomena using boron trichloride. We observed that the dehydro-β-carotene structure is especially sensitive to BF_3, much more so than is β-carotene. When BF_3-etherate is added to a

---

[1] Presented at the Meeting of the American Chemical Society, March 19, 1953, in Los Angeles, California.

[2] R. Kuhn and E. Lederer, *Ber.*, **65**, 637 (1932).

[3] P. Karrer and G. Schwab, *Helv. Chim. Acta*, **23**, 578 (1940).

[4] W. Oroshnik, G. Karmas and A. D. Mebane, THIS JOURNAL, **74**, 295 (1952).

[5] H. H. Strain, *ibid.*, **63**, 3448 (1941). "Boron trifluoride . . . converted carotenoids into unstable blue pigments . . . Alcohol reconverted these pigments into yellow substances that were strongly adsorbed on columns of magnesia and did not exhibit definite absorption maxima."

[6] G. N. Lewis and G. T. Seaborg, *ibid.*, **61**, 1886 (1939).