A small sample of the 2,2-dibromobutane was hydrolyzed in aqueous cuprous chloride to the corresponding ketone, 2-butanone, which was converted to its 2,4-dinitrophenylhydrazone, m.p. 117°.[22]

Reaction of the Complex with Dibutylamine.—A slurry of 160 g. (1.00 moles) of the complex in 200 ml. of nitroethane was cooled to 0° and 73.0 g. (0.565 mole) of dibutylamine was added slowly. The reaction stood 4 days at room temperature. The reaction was filtered and the organic liquid was washed with water, extracted with ether and the extract dried. The solvents were removed by vacuum distillation. The remaining solid was recrystallized from hot benzene, m.p. 258–260°. Mixture m.p. with a material prepared by bubbling gaseous boron trifluoride into dibutylamine in benzene, gave no depression. The total yield of the addition complex was 98%.

Reaction of the Complex with Ethyl Methylmalonate.—A slurry of 38 g. (0.238 mole) of the complex in 30 ml. of carbon tetrachloride was cooled to 0° and ethyl methylmalonate (40 g., 0.230 mole) was added dropwise within 2 hours. The reaction stood 3 days at room temperature and then was filtered, washed several times with water and dried. Fractionation yielded 10.1 g. of the starting ester and 23.6 g. (62.6%) of ethyl methylnitromalonate,[23] b.p. 66–67° (0.5 mm.), $n^{20}D$ 1.4361.

(22) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," third edition, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 262.

(23) W. Steinkopf and A. Supan, *Ber.*, **43**, 3245 (1910).

LAFAYETTE, INDIANA

[CONTRIBUTION NO. 2273 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

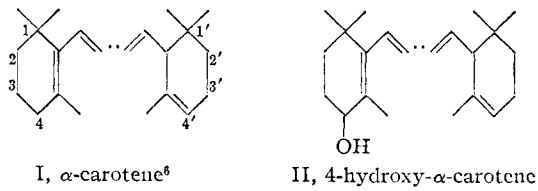
On Some Cleavage Products of the Boron Trifluoride Complexes of α-Carotene, Lycopene and γ-Carotene

BY WARREN V. BUSH AND L. ZECHMEISTER

RECEIVED DECEMBER 10, 1957

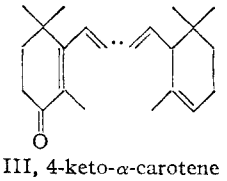
Hydrolysis of the deeply colored α-carotene–BF_3 complex yields mainly 4-hydroxy-α-carotene while the α-ionone end of the molecule remains unaltered. By dehydrogenation of the hydroxy compound 4-keto-α-carotene is obtained. Ethanolysis of the complex mentioned results in the formation of 4-ethoxy-α-carotene. Lycopene and γ-carotene were studied along similar lines. Upon cleavage of the complex, lycopene yielded 5,6-dihydroxy-5,6-dihydrolycopene, characterized by some conversions (Chart 2), while γ-carotene gave the 4-hydroxy compound. The cyclic terminal group of the γ-carotene complex appears to be more reactive than its acyclic end. Reaction mechanisms for the conversions mentioned are proposed (Charts 1, 3).

Although the formation of some deeply colored, rather sensitive carotenoid–boron halogenide complexes had been observed 18 years ago,[1] the cleavage products of such complexes were studied only recently, in our laboratory,[2–4] especially those obtained from the bicyclic, symmetrical β-carotene, $C_{40}H_{56}$, and some of its dehydrogenation products. We now have extended these experiments[5] to three structural isomers of β-carotene, *viz.*, the unsymmetrical, bicyclic α-carotene (I), the symmetrical, acyclic lycopene (XII) and the unsymmetrical, monocyclic γ-carotene (XXII).



I, α-carotene[6]

II, 4-hydroxy-α-carotene



III, 4-keto-α-carotene

α-Carotene.—When the dark blue complex of this polyene was treated with water, the original orange color of the carotene solution reappeared, and subsequent chromatographic analysis showed that about a third of the starting material had been converted into 4-hydroxy-α-carotene, $C_{40}H_{55}OH$ (II). This compound, although mentioned briefly before,[4] has been structurally clarified only in the present study. It is crystalline and forms crystalline alkyl ethers and an acetate. It shows the α-carotene spectrum (Fig. 1). The allylic position

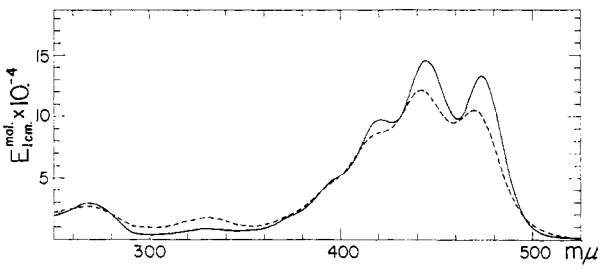


Fig. 1.—Molecular extinction curves (in hexane) of 4-hydroxy-α-carotene: ———, fresh solution of the all-*trans* compound; – – – –, mixture of *cis-trans* isomers after iodine catalysis.

of the OH– group was indicated by the positive reaction with a dilute, anhydrous HCl solution in chloroform,[7] in that a considerable deepening of the color was observed almost immediately and, upon chromatographing, 3,4-dehydro-α-carotene[3] (IV) was shown to be present. The same com-

(1) G. N. Lewis and G. T. Seaborg, THIS JOURNAL, **61**, 1886 (1939); H. H. Strain, *ibid.*, **63**, 3448 (1941).

(2) L. Wallcave, J. Leemann and L. Zechmeister, *Proc. Nat. Acad. Sci. U.S.*, **39**, 604 (1941).

(3) G. Karmakar and L. Zechmeister, THIS JOURNAL, **77**, 55 (1955).

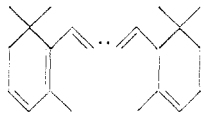
(4) F. J. Petracek and L. Zechmeister, *ibid.*, **78**, 3188 (1956).

(5) *Cf.* W. V. Bush, Thesis, California Institute of Technology, 1958.

(6) The two dots in the abbreviated formulas designate uninterrupted conjugation in an isoprenic structure.

(7) P. Karrer, *Helv. Chim. Acta*, **34**, 2160 (1951).

pound is obtained directly by the interaction of α-carotene and N-bromosuccinimide.[8]



IV, 3,4-dehydro-α-carotene

4-Hydroxy-α-carotene is autoxidizable, whereby the main point of attack is not the unsaturated system but the hydroxyl group. When air is bubbled through a chloroform solution, 10% of the material is converted into a new ketone, 4-keto-α-carotene (III), before extensive destruction takes place. The same carbonyl compound is formed as a (secondary) cleavage product of the α-carotene–BF_3 complex and, in better yields, by N-bromosuccinimide oxidation of either 4-hydroxy-α-carotene (II) or, indeed, of α-carotene itself. Lithium aluminum hydride reduces the ketone back to 4-hydroxy-α-carotene.

The conjugated position of the carbonyl group in the ketone III is confirmed by the shift of λ_{max} by 7 mμ (in hexane) toward longer wave lengths during the reaction II → III. Moreover, in contrast to the spectral curve of the hydroxy compound, that of the 4-keto-α-carotene is void of marked fine structure (Fig. 2).

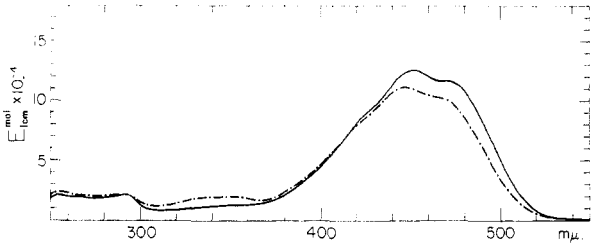


Fig. 2.—Molecular extinction curves (in hexane) of 4-keto-α-carotene: ———, fresh solution of the all-*trans* compound; – · – · –, mixture of *cis-trans* isomers after iodine catalysis.

The structural assignments mentioned are in accordance with infrared readings as well as with the lack of vitamin A effects in the rat of both II and III.

The ethanolysis of α-carotene–BF_3 leads to 4-ethoxy-α-carotene which can also be prepared by the addition of a trace of Karrer's acid chloroform reagent[7] to the anhydrous alcoholic solution of 4-hydroxy-α-carotene. On the other hand, the ethyl group is cleaved by acids in aqueous acetone. Like the corresponding hydroxy compound 4-ethoxy-α-carotene yields (in part) 3,4-dehydro-α-carotene (IV) when treated with the acid chloroform reagent.

Ammonolysis of α-carotene–BF_3 afforded (in part) 3,4-dehydro-α-carotene (IV) and an unidentified pigment that showed some interesting features and was also obtainable by hydrolysis of the complex (*cf.* Experimental part).

Assuming, as in the case of $SbCl_5$, that 1 mol. of C_{40}-carotenoid requires 2 mol. of BF_3 (while only 1 mol. is needed for vitamin A),[9,10] we believe that the limiting formulas of the resonating system in the α-carotene–BF_3 complex are V and VI (Chart 1) in

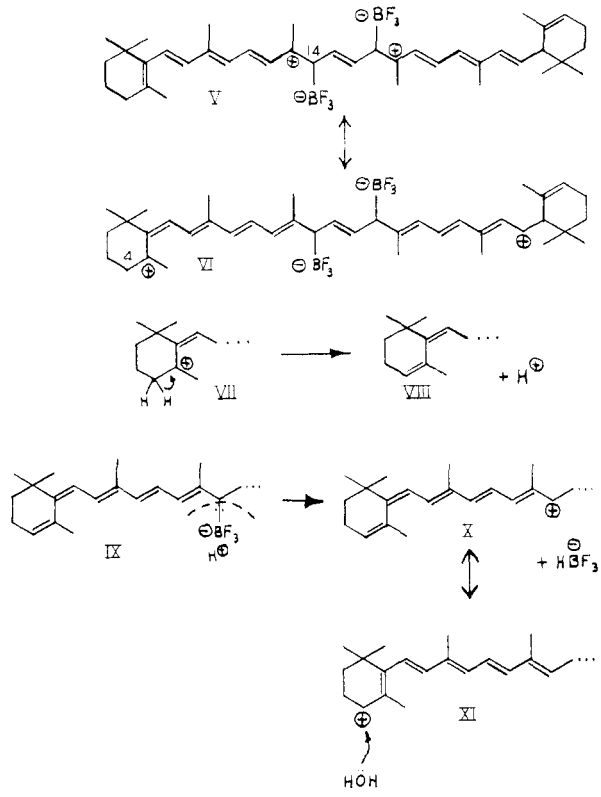


Chart 1.—Formation of 4-hydroxy-α-carotene from the α-carotene–BF_3 complex.

which the dark color is due to a resonating double carbonium ion (similar to that postulated by Meunier[9] for $SbCl_3$ (or $SbCl_5$) complexes). Hence, the chromophore effective in the cleavage process consists in reality of two shorter chromophores (here involving, respectively, four and three conjugated double bonds). The first step in this process may well be the removal of a proton from carbon 4 in VI and the formation of a 4,5-double bond, *i.e.*, the transition VII → VIII. The loss of an HBF_3^- ion at carbon 14 will then create there a carbonium ion that can transfer its charge by resonance to carbon 4, where, finally, interaction with water and loss of a proton would take place (IX→ XI), resulting in the formation of 4-hydroxy-α-carotene (II). Steric hindrance by the 1′-*gem*-dimethyl group seems to prevent any attack at that end of the α-carotene molecule.

Lycopene.—The hydrolysis of the dark blue lycopene–BF_3 complex yielded mainly a crystalline polyene, *viz.*, 5,6-dihydroxy-5,6-dihydrolycopene whose structure XIII follows from some conversions listed in Chart 2.

In this instance the chromophore indicated the presence of ten double bonds, and the spectrum

(8) G. Karmakar and L. Zechmeister, THIS JOURNAL, **77**, 55 (1955).

(9) P. Meunier and A. Vinet, "Chromatographie et mésomérie. Adsorption et résonance," Masson et Cie, Paris, 1947; P. Meunier, *Compt. rend.*, **215**, 470 (1942); *cf.* also L. Zechmeister and A. Sandoval, *Science*, **101**, 585 (1945).

(10) J. Brüggemann, W. Krauss and J. Tiews, *Naturwiss.*, **38**, 562 (1951); *Ber.*, **85**, 315 (1952).

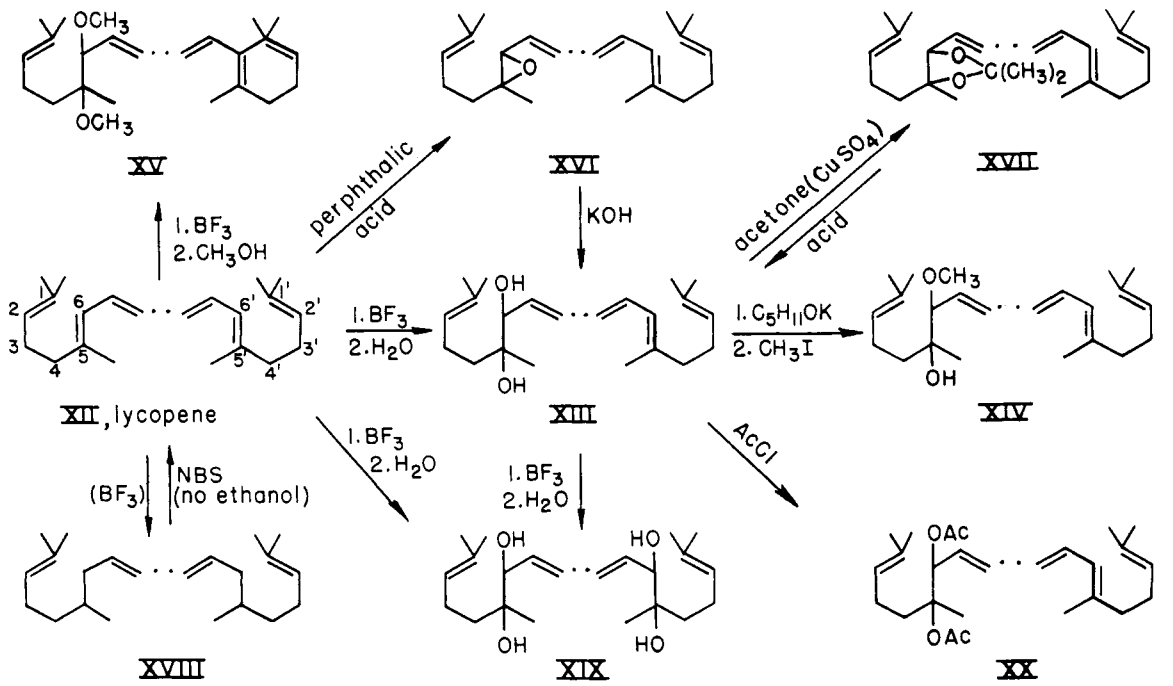
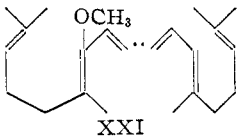


Chart 2.—Some conversions of 5,6-dihydroxy-5,6-dihydrolycopene (XIII).

(Fig. 3) was not altered by diacetylation. The α-glycol structure[11] of XIII was demonstrated by the formation of an acetonide (XVII). Furthermore, we have prepared from lycopene a small amount of lycopene mono-epoxide (XVI) (that does not seem to appear in the literature), and the latter yielded, upon an alkaline treatment, 5,6-dihydroxy-5,6-dihydrolycopene. That the two hydroxyl groups of the latter must be unequal, is brought out by the formation of a monoether only, under the conditions of the conversion XIII → XIV (Chart 2). If during the preparation of XIV the temperature applied was 100° (instead of 60°) much pigment suffered destruction; however, at the same time small quantities of a new monoether, showing the lycopene chromophore, appeared. We believe that this second methyl ether is a dehydration product of XIV and should be interpreted as 6-methoxylycopene (XXI).



XXI

Methanolysis of the lycopene–BF_3 complex yielded 5,6-dimethoxy-5,6-lycopene (XV), a compound possessing the same chromophore as XIII or XIV but clearly differentiated from these polyenes by its partition coefficient and chromatographic behavior.

Concerning the mechanism of the hydrolysis of the lycopene–BF_3 complex, we propose a route similar, in its initial steps, to that formulated above for α-carotene. 5,6-Dihydroxy-5,6-dihydrolycopene probably arises according to Chart 3 *via* the removal of a HBF_3^- ion.

(11) The two OH-groups of the natural product azafrin occupy positions identical with those in our dihydroxy compound, *cf.* R. Kuhn, A. Winterstein and H. Roth, *Ber.*, **64**, 333 (1931); R. Kuhn and A. Deutsch, *ibid.*, **66**, 883 (1933).

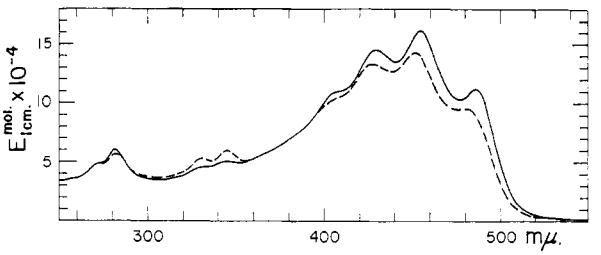
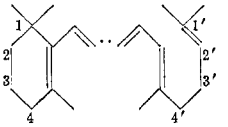


Fig. 3.—Molecular extinction curves (in hexane) of 5,6-dihydroxy-5,6-dihydrolycopene: ———, fresh solution of the all-*trans* compound; – – – –, mixture of *cis-trans* isomers after iodine catalysis.

It is reasonable to assume that a by-product of the cleavage process, *viz.*, 5,6,5′,6′-tetrahydroxy-5,6,5′,6′-tetrahydrolycopene (XIX) (Fig. 4), results from simultaneous hydrolysis at both ends of a complex, involving two molecules of BF_3 but only one lycopene, similar to the 2:1 complexes proposed by Meunier[9] and Brüggemann[10] for Lewis acids and α- or β-carotene. Our tetraol XIX was also obtained by hydrolyzing 5,6-dihydroxy-5,6-dihydrolycopene–BF_3 (Chart 3).

γ-Carotene.—Because of its monocyclic nature, this rare carotene (XXII) was expected to behave in part as "β-carotene" and in part as "lycopene." Indeed, the mixture obtained upon hydrolyzing γ-carotene–BF_3 showed unusual complexity. Among



XXII, γ-carotene

the crystallizable products the relatively highest

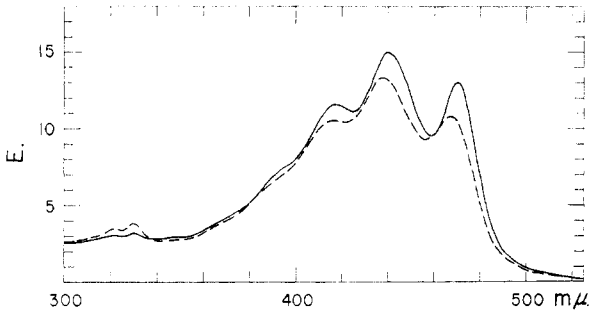


Fig. 4.—Extinction curves (in hexane) of 5,6,5′,6′-tetrahydroxy-5,6,5′,6′-tetrahydrolycopene: ———, fresh solution of the all-*trans* compound; – – – –, mixture of *cis–trans* isomers after iodine catalysis.

yields (2–3%) were obtained of a pigment that showed a spectrum very similar to that of γ-carotene (Fig. 5) and was identified as 4-hydroxy-γ-carotene in the following manner. It was etherified readily when submitted to acid catalysis in alcohol at 20° and it was easily dehydrated by means of the acid chloroform reagent. Therefore the hydroxyl group is in an allylic position (either 4 or 4′); this was confirmed by a considerable spectral shift toward longer wave lengths when the hydroxy compound was oxidized by air to the corresponding ketone; hence, the carbonyl group of the latter must be a part of the chromophore.

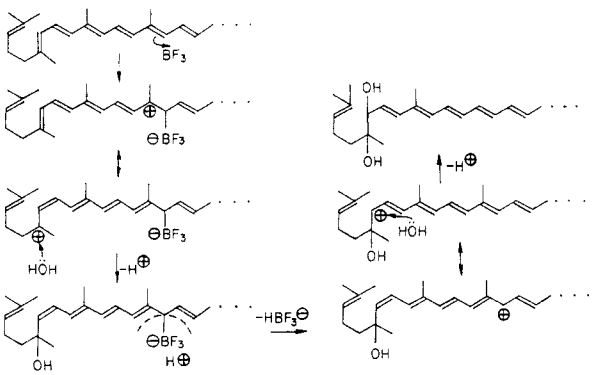
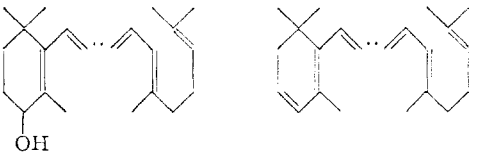


Chart 3.—5,6-Dihydroxy-5,6-dihydrolycopene from lycopene.

The 4′-position for the OH– group has been excluded on the following two grounds: (a) Such a position would cause, after dehydration, a lengthening of the conjugated system by two double bonds, since then the new double bond would connect the isolated 1′,2′-double bond with the main chromophore; the observed spectral shift of 9 mμ was, however, too small compared with the expected effect of two additional double bonds. (b) According to earlier observations[12,13] the introduction of a second conjugated double bond into a β-ionone ring involves the loss of fine structure in the main spectral band, in contrast to the continued presence of sharp fine structure after a similar operation at the aliphatic end group. Since after dehydration our compound did not show any fine structure, it must be the 4-hydroxy derivative XXIII and its dehydration product is XXIV.



XXIII, 4-hydroxy-γ-carotene

XXIV, 3,4-dehydro-γ-carotene

We may state in summary that BF_3 complexing and subsequent hydrolysis affect mainly one terminal group of the four natural products, α-carotene, β-carotene, γ-carotene and lycopene. In case the molecule contains one β-ionone ring only, this is the preferred site of the substitution.

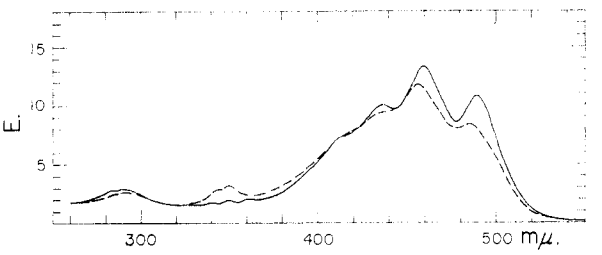


Fig. 5.—Extinction curves (in hexane) of 4-hydroxy-γ-carotene: ———, fresh solution of the all-*trans* compound; – – – –, mixture of *cis–trans* isomers after iodine catalysis.

Experimental

Materials and Methods.—α-Carotene was prepared from commercial carotene (Barnett Lab., Long Beach, Calif.), lycopene from tomato paste,[14] and γ-carotene from pro-γ-carotene by iodine catalysis.[15]

When no other adsorbent is mentioned, calcium hydroxide (Sierra Hydrated Lime, Superfine, U. S. Lime Products Corp., Los Angeles, Calif.) was used mixed with 0.5 part (by weight) of Celite (No. 545, Johns–Manville Co.). This mixture will be termed "lime–Celite." Other adsorbents: magnesium oxide (Sea Sorb 43; Food Machinery and Chemical Corp., San Jose, Calif.), alumina (Alorco, Grade F; Aluminum Ore Co., East St. Louis, Ill., −80 mesh, reground to −200 mesh), and zinc carbonate (powdered technical; Harshaw Chem. Co., Los Angeles, Calif.). Unless other dimensions are given, 45 × 4.5 cm. columns were applied. For development of chromatograms, hexane or a hexane–acetone mixture or (for lycopene) a hexane–chloroform mixture was used. Pigment zones were cut out and eluted with acetone.

Alcohol-free chloroform was obtained by washing, drying and distilling over P_2O_5; and alcohol-free acetone, by refluxing for 4 hr. with suspended permanganate. "Optical" hexane was prepared by treating commercial hexane repeatedly with fuming sulfuric acid (65% SO_3) and distilling. Eastman Spectro Grade chloroform and carbon tetrachloride were used for determining infrared spectra.

Boron trifluoride etherate was prepared according to Hennion, *et al.*[16]: 440 g. of the gas (Ohio Chemical and Manufacturing Co., Cleveland, Ohio) was dissolved in the same weight of anhydrous ether (reagent grade) while stirring and cooling in an ice-bath. The solution was distilled through a short column packed with 5-mm. glass beads and the 124–125° (cor.) fraction was stored in a dark bottle (all-glass apparatus, silicone stopcock grease, Carborundum boiling chips). All evaporations were carried out *in vacuo* at 55°, in a slow stream of nitrogen. Hexane or chloroform

(12) F. J. Petracek and L. Zechmeister, THIS JOURNAL, **78**, 1427 (1956).

(13) F. J. Petracek, Thesis, California Institute of Technology, 1956.

(14) A. Sandoval and L. Zechmeister, "Biochemical Preparations," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1949, p. 57.

(15) L. Zechmeister and J. H. Pinckard, THIS JOURNAL, **69**, 1930 (1947).

(16) G. F. Hennion, H. D. Hinton and J. A. Nieuwland, *ibid.*, **55**, 2857 (1933).

solutions were washed in the automatic LeRosen apparatus[17] and dried over sodium sulfate.

In order to be recrystallized, a sample was dissolved at 40° in the minimum amount of benzene, whereupon abs. or 95% methanol was added cautiously, dropwise, running down the glass wall, while stirring. The liquid was maintained at 40° until crystallization was almost complete, and then kept at 4° for a day. The crystals were centrifuged, washed with a small amount of the second solvent, centrifuged again and dried *in vacuo*, in an Abderhalden apparatus at the temperature of refluxing acetone for 2 hr.

Melting points (cor.) were taken in evacuated and sealed capillaries in an electrically heated Berl block. Partition ratios were determined as described recently[18] and refer to hexane–95% methanol.

Visible and ultraviolet spectra were obtained with a Cary recording spectrophotometer, model 11M (10-mm. cells); the molar extinction values represent the average of results based on two independent weighings. For visual observations a Loewe–Schumm evaluating grating spectroscope (Zeiss) was used. The spectral maxima given refer to hexane solutions. Infrared spectra were recorded in a Perkin–Elmer infrared spectrophotometer, model 21 (1% solutions in 1.0-mm. NaCl cells; NaCl prism).

Iodine Catalysis.—After the extinction of the solution had been measured, about 1% of the pigment weight of iodine (1 drop of a 0.1% solution in opt. hexane) was added to the solution in the 3-ml. spectrophotometer cell. The solution was then illuminated for 10 min. (two 3500° Mazda fluorescent lamps, 40 watt, 120 cm. long, white and yellowish; distance, 60 cm.).

Test for Allylic Hydroxyl or Alkoxyl Groups in Carotenoids.—To a few ml. of a chloroform solution (3 mg. of pigment per l., contained in a 150 × 18 mm. test-tube) 5 drops of a saturated chloroform solution of dry HCl gas was added. The test was considered as positive if marked deepening of the color was observed within a few minutes.

Microanalyses were performed by Dr. A. Elek, Los Angeles, Calif.; by Mr. G. Swinehart, Pasadena, Calif.; and by Microchemical Specialties Co., Berkeley, Calif.

Vitamin A assays were carried out by the late Dr. H. J. Deuel, Jr., and by Mr. A. Wells, both of the University of Southern California in Los Angeles.

α-Carotene. **Formation and Cleavage of the α-Carotene–BF_3 Complex.**—A total of 3 g. of α-carotene, divided into thirty portions, was treated as follows. To each 100-mg. portion (in 100 ml. of chloroform) 10 ml. of BF_3-etherate was added with stirring. After 120–150 sec. of total complexing time, *i.e.*, exactly 60 sec. after the solution had turned from a brownish-green to deep blue, it was poured rapidly, with agitation, into 220 ml. of 90% acetone (0°); the original red-orange color reappeared. The liquid was then poured into a 1-l. separatory funnel containing 150 ml. of saturated bicarbonate and 100 ml. of iced water. After thorough shaking the two layers separated; the lower (chloroform) phase was poured into 1 vol. of acetone, shaken, and washed acetone-free. This operation was considered to be complete when the volume of the hypophase had been reduced to 100 ml., whereupon it was dried and evaporated. The residue was dissolved in 25 ml. of hexane and developed with 750 ml. of hexane for about 45 min. (the figures on the left denote the width of zones, in mm.; the lines indicate the points where the lettered zones were cut out).

42 orange, narrow brown bands at top 3 red 9 yellow 26 light yellow	zone R
5 colorless interzone 10 light yellow	
32 yellow	zone Q
73 six minor zones and interzones	
27 yell.: unchanged α-carotene (2.7 mg.)	zone T
23 pale yellow 10 colorless interzone 10 very pale yellow	
30 yellow (*cf.* footnote 22)	zone B
18 pale yellow 14 colorless interzone	
28 pale green	zone G

Filtrate: blue-grey fluorescent in ultraviolet light

After elution, the respective pigment fractions were transferred to hexane by means of water; the solutions were then washed acetone-free and dried. The solution originating from zone R was evaporated to 25 ml. and developed with about 750 ml. of hexane + 4% acetone.

44 several minor zones 22 nearly colorless interzone	
50 yellow	zone A
8 nearly colorless interzone	
82 orange-yellow	zone C
3 pink 6 yellow 25 orange-yellow	zone D
13 red 4 orange	zone E
77 sev. minor zones and interzones	
16 yellow 24 yellow	zone H[19]

The lettered hexane solutions were stored in the refrigerator in filled volumetric flasks until all thirty corresponding solutions were ready.

4-Hydroxy-α-carotene.—The combined thirty solutions C were evaporated to 300 ml. and developed for 4 hr. on two 60 × 8 cm. columns, using 2 × 3 l. of hexane + 3% acetone.

30 several tan zones
60 colorless interzone
30 pale yellow (neo-4-hydroxy-α-carotene V, etc.)
60 colorless interzone
250 orange (all-*trans*-4-hydroxy-α-carotene)
50 yellow (neo-4-hydroxy-α-carotene A, etc.)
90 a red and a pink zone

The eluate of the 250-mm. zone (after transfer to hexane) was evaporated completely and crystallized from benzene–95% methanol; photometrically estimated yield, 630 mg. or 21% of the α-carotene. The crystals weighed 285 mg. (9.5%); maxima 474, *445*, 421 mμ; E^{mol}_{1cm} 14.5 × 10^4 at λ_{max} (*cf.* Fig. 1). A sharp O–H stretching band appeared at 2.76 μ (in CCl_4); large, rectangular orange plates with split ends, m.p. 177–178°; partition ratio, 84:16 in hexane–95% methanol. The compound is adsorbed on lime–Celite above 4-keto-α-carotene. The allylic reaction is positive but weaker than with isocryptoxanthin. 4-Hydroxy-α-carotene shows no vitamin A effect in the rat.

Anal. (two independent samples): Calcd. for $C_{40}H_{56}O$: C, 86.89; H, 10.21. Found: C, 86.42, 86.88; H, 10.17, 11.10.

By fractionating zones A and D six *cis* forms were obtained in chromatographically homogeneous but not crystalline state; two of them were adsorbed above the all-*trans* compound.

4-Acetoxy-α-carotene.—To a solution of 25 mg. of 4-hydroxy-α-carotene in 0.3 ml. of anhydrous pyridine 2 drops of freshly distilled acetyl chloride (C.P.) was added. The pasty mixture was stirred and kept at 20° for 0.5 hr., whereupon 1.7 ml. of abs. methanol was introduced. The colorless crystals of the pyridine–acetyl chloride adduct dissolved rapidly, while the acetate slowly crystallized. The long, dark red prisms were washed twice with methanol;

(17) A. L. LeRosen, *Ind. Eng. Chem., Anal. Ed.*, **14**, 165 (1942).

(18) F. J. Petracek and L. Zechmeister, *Anal. Chem.*, **28**, 1484 (1956).

(19) Upon rechromatography of the combined thirty zones H, 3 mg. of crystalline "unidentified pigment II" was isolated: irregular clusters of orange-yellow platelets, m.p. 177–178° (sintered at 173°); partition ratio, 94:6. This pigment adsorbed below 4-keto-α-carotene but slightly above 4-ethoxy-α-carotene.

yield 20.6 mg. (82%), m.p. 159–161°; partition ratio, 99:1; allylic test, positive; maxima 474, *444*, 421 mμ. The acetate is adsorbed below 4-keto-α-carotene but above 4-ethoxy-α-carotene.

Anal. Calcd. for $C_{42}H_{58}O_2$: C, 84.80; H, 9.83. Found: C, 85.23; H, 10.28.

"Unidentified Pigment I."—The combined hexane solutions of the thirty zones E were evaporated to 50 ml. and developed on two columns, with two 750-ml. portions of hexane + 3% acetone.

24 colorless section
205 five pink and orange zones, and interzones
43 red ("unidentified pigment I")
18 yellow
13 pinkish orange
77 red (all-*trans*-4-keto-α-carotene)
10 yellow
19 colorless interzone
41 orange

The solution of the unidentified pigment was evaporated and the residue crystallized from benzene–methanol; photometrically estimated yield, 8 mg. (0.3%); weight of the crystals, 4 mg. (0.1%); small, deep red oval platelets, m.p. 184–186°; partition, ratio, 94:6; maxima 503, *472*, 442 mμ (Fig. 6). On lime–Celite this pigment is adsorbed somewhat above 4-keto-α-carotene, below 4-hydroxy-α-carotene and much below *retro*-dehydrocarotene; allylic test, negative.

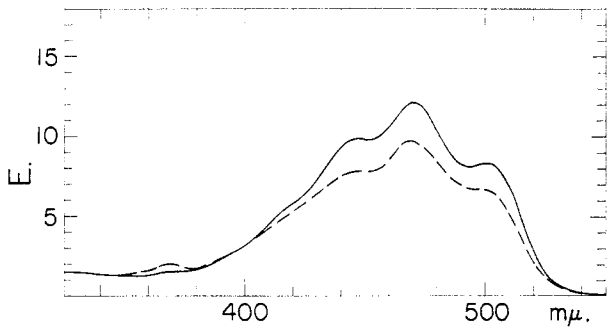


Fig. 6.—Extinction curves (in hexane) of "unidentified pigment I": ———, fresh solution of the all-*trans* compound; – – – –, mixture of *cis–trans* isomers after iodine catalysis.

4-Keto-α-carotene. (a) **From the BF_3 Complex** (*cf.* above).—The ketone was eluted from the 77-mm. zone (see above) and crystallized from benzene–methanol; photometrically estimated yield, 19.3 mg. (0.7%); weight of the crystals, 14.6 mg. (0.5%); salmon-pink, "toy boat"-like platelets, m.p. 188–189°; partition ratio, 91:9; single maximum in hexane at 452 mμ, $E_{1cm}^{1\%}$ 12.6 × 10^4 (Fig. 2). A strong carbonyl band was observed at 6.04 μ (in $CHCl_3$). The ketone is adsorbed on lime–Celite below 4-hydroxy-α-carotene but above 4-methoxy-, 4-ethoxy- or 3,4-dehydro-α-carotene. In a mixed chromatogram test the sample did not separate from a preparation obtained by oxidation of α-carotene with N-bromosuccinimide; allylic test, negative; vitamin A bioassay, negative.

Anal. Calcd. for $C_{40}H_{54}O$: C, 87.21; H, 9.88. Found: C, 87.35; H, 10.83 (corrected for 1.4% ash).

(b) **From 4-Hydroxy-α-carotene.**—Fifty mg. of substance was dissolved in 15 ml. of reagent grade chloroform (0°) which had been made alcohol-free immediately prior to use by washing with water and distilling, in darkness, over P_2O_5. An ice-cold solution of 16 mg. of N-bromosuccinimide in 15 ml. of alcohol-free chloroform was added while the solution was being violently agitated by a nitrogen stream. One minute later, 33 mg. of powdered N-phenylmorpholine was introduced and the agitation continued for 2 more min. The liquid was then refluxed for 15 min., washed with sodium bicarbonate, then for 15 min. with water, dried, and developed with 750 ml. of hexane + 4% acetone.

125 several pale zones
20 colorless interzone
90 pale salmon-pink
22 colorless interzone
97 salmon-pink (4-keto-α-carotene)
74 two yellow zones

The 4-keto-α-carotene was rechromatographed (column, 32 × 3.5 cm., developer, hexane + 3% acetone). The yield was 12.5 mg. (25%) as established photometrically; the crystals (from benzene–methanol) weighed 9.2 mg. (18%).

(c) **From α-Carotene and N-Bromosuccinimide.**—To a solution of 200 mg. of α-carotene in 20 ml. of reagent grade chloroform (−17°) was added, with violent agitation (nitrogen stream), 132 mg. of NBS in 20 ml. of chloroform (−17°). The reaction was allowed to continue for 60 sec. (not longer!), whereupon 264 mg. of N-phenylmorpholine crystals were introduced; agitation was continued for 1 more min., followed by 15 min. refluxing. After washings with sodium bicarbonate and water, the dried solution was evaporated completely and developed on two 30 × 8 cm. columns with two 750 ml. portions of hexane + 3% acetone.

70 several brown, tan and pink zones
19 colorless interzone
43 pink (4-keto-α-carotene)
68 two light orange zones
22 colorless interzone
25 orange (3,4-dehydro-α-carotene, 30.5 mg.)
10 yellow

The yield of 4-keto-α-carotene was 67.2 mg. (34%, photometrical data); the crystals weighed 17 mg. (8.5%).

4-Keto-α-carotene 2,4-Dinitrophenylhydrazone.—To a solution of 9 mg. of 4-keto-α-carotene (*ex* complex) in 2 ml. of benzene (reagent grade) first 4 ml. of abs. ethanol was added cautiously, with stirring, and then 2 ml. of the Shriner and Fuson 2,4-dinitrophenylhydrazine reagent.[20] After standing for 0.5 hr., the centrifuged microcrystals were washed once with a few ml. of abs. ethanol, then twice with hexane, and dried for 2 hr.; yield 9.8 mg. (81%), m.p. 216–217°.

Anal. Calcd. for $C_{46}H_{58}O_4N_4$: N, 7.67. Found: N, 7.19.

Reduction of 4-Keto-α-carotene to 4-Hydroxy-α-carotene.—Twenty-five mg. of substance was dissolved in 50 ml. of a 9:1 mixture of anhydrous ether and benzene, cooled to 0° and poured quickly, with swirling, into an ice-cold suspension of 100 mg. of $LiAlH_4$ in 100 ml. of anhydrous ether. This mixture was kept at 0° for 15 min. and the excess reagent was decomposed by dropwise addition of methanol (0°). The liquid, containing much gelatinous precipitate, was washed until the effluent had *p*H 6–7. The dried solution was then developed with hexane + 4% acetone. Only a single main yellow zone, containing 4-hydroxy-α-carotene, appeared; yield 22 mg. (88%, photometric); weight of crystals, 16 mg. (64%). In a mixed chromatogram test the sample did not separate from 4-hydroxy-α-carotene *ex* α-carotene–BF_3.

3,4-Dehydro-α-carotene from 4-Hydroxy-α-carotene.—To a solution of 15 mg. of the hydroxy compound in 20 ml. of reagent grade chloroform 10 drops of the acid chloroform reagent was added; 10 min. later the solution was diluted with 50 ml. of hexane, washed thoroughly with bicarbonate and water, dried, evaporated, redissolved in 25 ml. of hexane and developed with 800 ml. of the same solvent.

160 several yellow, tan and orange zones
60 light yellow (4-ethoxy-α-carotene, 2 mg.)
40 two pale yellow zones
68 orange (3,4-dehydro-α-carotene)
77 several *cis* forms of the dehydro compound

The photometrically established yield (including *cis* isomers) was 9 mg. (60%); the all-*trans* crystals obtained from benzene–methanol weighed 3.2 mg. (21%).

Ethanolysis and Methanolysis of the α-Carotene–BF_3 Complex.—The complex prepared from 100 mg. of α-carotene was cleaved by the addition, with swirling, of 200 ml. of abs. ethanol (4°). The solution was washed, dried, evaporated, and developed with 1 l. of hexane.

(20) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 171.

46 several pink, tan and yellow zones
41 colorless interzone
56 yellow, heterogeneous (*cis*-4-ethoxy-α-carotenes)
39 colorless interzone
118 yellow (all-*trans*-4-ethoxy-α-carotene)
35 light yellow (*cis*-4-ethoxy-α-carotenes)
7 pink-orange
12 yellow (unchanged α-carotene, 9 mg.)
10 colorless interzone
24 yellow (unidentified)

The complex of another 100-mg. portion of α-carotene was cleaved with 200 ml. of abs. methanol. The subsequent chromatogram was similar to that obtained on ethanolysis.

4-Ethoxy-α-carotene.—The photometrically established yield after ethanolysis of the complex amounted to 16 mg. (16%) of all-*trans*-4-ethoxy-α-carotene (crystals, 10 mg.), and 24 mg. of the *cis* forms. The same ethoxy compound appeared also, in much lower yields, upon hydrolysis of the complex when the ingredients used were not absolutely ethanol-free. Thus the hexane solution originating from the thirty zones Q (see above) was concentrated to 100 ml. and developed with 2 l. of hexane on a 60 × 8 cm. column.

75 several tan and pale yellow zones and interzones
135 colorless interzone
155 yellow (all-*trans*-4-ethoxy-α-carotene)
25 light yellow (a *cis*-4-ethoxy-α-carotene)
5 colorless
45 two pale yellow zones (*cis* forms)

The all-*trans*-4-ethoxy-α-carotene was crystallized from benzene–methanol; yield 87 mg. (3%) of the α-carotene as estimated photometrically; the crystals (from benzene–methanol) weighed 69 mg. (2.3%); clusters of oval or pin-shaped, dull yellow-orange platelets, m.p. 176–177°; partition ratio, 99:1; maxima 473, 444, 420 mμ; $E^{mol}_{1\,cm}$ 14.3×10^4 at λ_{max}. The compound is adsorbed above α-carotene and even somewhat above β-carotene but below 4-keto-α-carotene. It did not separate from a sample obtained by ethanolysis of the α-carotene–BF_3 complex; allylic test, positive; vitamin A test, negative.

Anal. Calcd. for $C_{40}H_{55}(OC_2H_5)$: C, 86.83; H, 10.41; OC_2H_5, 7.75. Found: C, 86.68; H, 10.13; OC_2H_5, 7.62 (cor. for 2.0% ash).

4-Ethoxy-α-carotene was also obtained by direct etherification as follows: To a solution of 25 mg. of 4-hydroxy-α-carotene in 5 ml. of reagent grade chloroform were added 25 ml. of abs. ethanol and 10 drops of the acid chloroform reagent. Five minutes later the solution was diluted with 25 ml. of hexane and washed first with sodium bicarbonate, then thoroughly with water. After drying, it was developed with hexane + 2% acetone. An upper, minor zone was that of unchanged starting material, and the lower, main yellow zone contained the desired ether; yield 9 mg. (36%). The ethyl ether could be reconverted with 40% yield into 4-hydroxy-α-carotene by a procedure very similar to that described when water was used instead of alcohol and just enough acetone to produce a single phase.

4-Methoxy-α-carotene was isolated upon methanolysis of the BF_3-complex (see above); photometrically established yield, 40 mg. (40%, including *cis* forms); crystals, 10 mg.; oval, dull yellow-orange platelets (from benzene–methanol), m.p. 170–171°; partition ratio, 99:1; maxima 474, *445*, 421 mμ. The compound is adsorbed below 4-keto-α-carotene but considerably above α-carotene and slightly above 4-ethoxy-α-carotene; allylic test, positive.

Anal. Calcd. for $C_{40}H_{55}(OCH_3)$: OCH_3, 5.47. Found: OCH_3, 4.91.

An identical product was obtained by direct etherification of 4-hydroxy-α-carotene under the conditions given for the ethoxy compound; yield from 25 mg. starting material, 13.6 mg. (54%). For reconversion into the hydroxy derivative see above.

Ammonolysis of the α-Carotene–BF_3 Complex.—Since preliminary experiments in which reagent grade chloroform was used resulted in the formation of much 4-ethoxy-α-carotene, the chloroform was dealcoholated and dehydrated immediately prior to use, by gravity filtration through a 18 × 3.5 cm. activated alumina column (Woelm, Basic, Activity Grade I).[21] To 90 mg. of α-carotene in 100 ml. of chloroform 9 ml. of BF_3-etherate was added. The dark liquid was swirled rapidly, kept at room temperature for exactly 2 min., whereupon 40 ml. of a saturated solution of anhydrous ammonia in alcohol-free chloroform was introduced; the original orange-red color was thus restored. (A greenish-white precipitate appeared.) The liquid was washed, dried, evaporated, and redissolved in hexane; recovery of pigment, 2–3%. The substance was developed on a 24 × 4.5 cm. column with 250 ml. of hexane.

127 several pigments and interzones
20 orange (3,4-dehydro-α-carotene)
29 yellow (unchanged α-carotene, 3 mg.)
12 colorless interzone
20 yellow orange (unidentified)[22]
8 yellow

The 3,4-dehydro-α-carotene (2 mg., 2.2%) was identified by comparison with an authentic sample obtained by the interaction of α-carotene and N-bromosuccinimide.[8] *retro*-Dehydrocarotene was absent.

Lycopene. **Formation and Cleavage of the Lycopene–BF_3 Complex.**—The complexing of a total of 8 g. of pigment was carried out in eighty portions. To a solution of 100 mg. of lycopene in 50 ml. of alcohol-free chloroform (0°) 3 ml. of the etherate (20°) was added, with agitation, in an ice-bath. After exactly 30 sec. the greenish-brown solution was poured quickly, with swirling, into a precooled mixture of 180 ml. of alcohol-free acetone + 20 ml. of saturated bicarbonate solution. After 10 sec. the orange color was restored and the mixture was then allowed to assume room temperature. Twenty such portions were combined, washed continuously, in darkness for 2 hr., dried, concentrated to 100 ml., diluted with hexane to 1 l. (photometrically measured pigment recovery, 50% "lycopene"). The solution was developed on twenty 30 × 8 cm. columns with about 1 l. of chloroform–hexane (2:3) each (a total of eighty columns was required).

46 several brown yellow and pink zones
14 light yellow (zone 0)
70 many pale orange and pink zones
10 colorless interzone
46 orange (zone I)
24 yellow (zone II)
21 pink (unchanged lycopene, 14%) (zone III)
24 several orange zones (zone IV)
Filtrate, yellow ("zone" V)

Each numbered zone was eluted and transferred to a chloroform–hexane mixture (2:3) by addition of water. After dilution with 1 vol. of hexane, the solutions were washed, dried, and stored at 4°.

5,6-Dihydroxy-5,6-dihydrolycopene.—The combined eighty solutions originating from zones I (see above) were evaporated to 500 ml. and developed on four columns with 1 l. of chloroform–hexane 2:3 each.

108 sev. narr., pale yell. and tan zones and interzones
11 colorless interzone
80 bright orange (5,6-dihydroxy-5,6-dihydrolycopene)
22 yellow (*cis* isomers of the diol)
27 colorless interzone
10 orange

After elution, transfer into chloroform–hexane and evaporation, the 5,6-dihydroxy-5,6-dihydrolycopene was crystallized from benzene–95% methanol (the *cis* isomers were combined with zone II); photometrically established yield, 604 mg. (7.5% of 8 g. of lycopene); the crystals weighed 337 mg. (4%); long, thin, needle-like, orange prisms (from benzene–95% methanol), m.p. 170–171°; partition ratio, 45:55 in hexane–95% methanol and 76:24 in hexane–90% methanol; maxima 488, *456*, 431 mμ; $E^{mol}_{1\,cm}$ 16.1×10^4 at λ_{max} (Fig. 3). A sharp hydroxyl band appeared at 2.78 μ, approximately twice as strong as that observed for 4-hydroxy-α-carotene (in CCl_4). The pigment was adsorbed on lime–Celite above lycopene but below lycoxanthin; reaction with acid chloroform, negative.

Anal. Calcd. for $C_{40}H_{58}O_2$: C, 84.15; H, 10.24; active hydrogen, 2.0. Found: C, 84.20, 83.92; H, 10.56, 10.14

(21) G. Wohlleben, *Angew. Chem.*, **68**, 752 (1956).

(22) This remarkable pigment (zone B, see above) showed the β-carotene spectrum, although it was adsorbed considerably below α-carotene.

(cor. for 1.7% ash[23]); active hydrogen (Zerevitinov), 2.1.

Diacetate.—To 9 mg. of 5,6-dihydroxy-5,6-dihydrolycopene in 0.3 ml. of anhydrous pyridine 10 drops of acetyl chloride (C.P.) was added, while stirring and cooling; the mixture was then refluxed for an hour. The pigment was transferred to hexane, washed continuously for 30 min., and developed with 250 ml. of chloroform–hexane 1:5 on zinc carbonate–Celite 6:1 (20 × 3.5 cm.). The upper main zone contained unreacted starting material and the lower one, the diacetate (3 mg.); partition ratio, 89:11 in hexane–95% methanol; maxima 487, *455*, 428 mμ; reaction with acid chloroform, negative.

Acetonide.—To 25 mg. of 5,6-dihydroxy-5,6-dihydrolycopene, in 25 ml of anhydrous benzene, 25 ml. of anhydrous acetone and 50 mg. of anhydrous copper sulfate were added. The solution was shaken in darkness for 16 hr., washed acetone-free, and evaporated. The residue, redissolved in hexane, was developed on a 20 × 3.5 cm. column with 500 ml. of chloroform–hexane 1:9. The main zone and two minor orange ones included unchanged starting material and its *cis* isomers, while a minor yellow zone located near the bottom contained 0.6 mg. (2.4%) of the acetonide; partition behavior, 97:3 in hexane–95% methanol; maxima 486, *455*, 429 mμ. On lime–Celite the compound is retained slightly above 5,6-dimethoxy-5,6-dihydrolycopene; reaction with acid chloroform, negative. The acetonide can be reconverted into 5,6-dihydroxy-5,6-dihydrolycopene with 1 ml. of *N* HCl in 10 ml. of abs. alcohol solution at 20°.

5,6-Dimethoxy-5,6-dihydrolycopene.—Each of two 125-mg. portions of lycopene was treated with 15 ml. of BF_3 etherate; the complex was cleaved with 600 ml. of abs. methanol at 0° as described above. The product was developed on a 100 × 8 cm. column with 4 l. of chloroform–hexane (1:9) for 6 hr.

10 brown
125 sev. colored zones (unchanged lycopene and *cis* forms; 33 mg.)
47 colorless interzone
118 bright yell.-orange (all-*trans*-5,6-dimethoxy-5,6-dihydrolycopene)
165 yellow (*cis* isomers of the latter)

The yield (including *cis* forms) was 65 mg. (26%) and the weight of crystals (from benzene–95% methanol) 20 mg. (8%); long, flat, orange-yellow prisms, in part clustered, m.p. 153–154°; partition ratio, 98:2 in hexane–95% methanol. The spectrum was similar to that of the dihydroxy compound (487, *456*, 430 mμ). On lime–Celite the dimethyl ether was adsorbed below neolycopene A but above β-carotene; reaction with acid chloroform, negative.

Anal. Calcd. for $C_{40}H_{56}(OCH_3)_2$: OCH_3, 10.32. Found: OCH_3, 9.33.

5,6,5′,6′(?)-Tetrahydrolycopene.—The combined eighty chromatographic filtrates ("zones" V, see above) were, after concentration to 1 l., developed on ten 30 × 8 cm. columns with 1 l. of hexane each. The main yellow zone was crystallized from benzene–methanol; yield 9.8 mg. (1.2%) (photometric estimate); the crystals weighed 2 mg. (0.3%); long, hair-like, light-yellow needles, occasionally forming a "fur ball," m.p. 137–139° (sintered at 135°); partition ratio 100:0 in hexane–95% methanol; maxima, 473, *441*, 418 mμ. The compound is adsorbed on lime–Celite below α-carotene; reaction with acid chloroform, negative.

Dehydrogenation to Lycopene.—Fifteen mg. of the tetrahydrolycopene was treated with N-bromosuccinimide as described for 3,4-dehydro-α-carotene. A subsequent chromatogram (20 × 3.5 cm.; chloroform–hexane 1:5) yielded, among others, a red zone containing 0.1 mg. of lycopene.

5,6,5′,6′-Tetrahydroxy-5,6,5′,6′-tetrahydrolycopene. (a) **From Lycopene.**—The eighty combined Zones O (see above) were evaporated to 100 ml. and developed on a single column with 2 l. of chloroform + hexane 1:1. The main zone contained 10 mg. of the compound (0.1%); and 2 mg. of crystals were obtained from chloroform–hexane; very small, nearly rectangular, yellow-orange prisms, arranged in clusters (from chloroform–hexane), m.p. 144–147° (sintering at 140°); partition ratio, 0:100 in hexane–95% methanol, 4:96 in hexane–90% methanol and 8:92 in hexane–85% methanol. The compound is adsorbed on lime–Celite above lycoxanthin (monohydroxy-lycopene); reaction with acid chloroform, negative; maxima, 470, *440*, 417 mμ. This spectrum indicates the presence of nine conjugated double bonds similar to that of "Compound F" obtained by partial hydrogenation of lycopene.[24] An extremely intense hydroxyl absorption was observed at 2.85 μ (Nujol mull). Furthermore, the partition behavior strongly supports the polyhydroxy structure.

(b) **From 5,6-Dihydroxy-5,6-dihydrolycopene.**—Complexing 25 mg. of substance as described for lycopene, hydrolyzing the complex at 20°, and developing on a 24 × 4.5 cm. column with 1 l. of chloroform–hexane 2:3 resulted in the isolation of 2 mg. of 5,6,5′,6′-tetrahydroxy-5,6,5′,6′-tetrahydrolycopene from the single main zone.

5-Hydroxy-6-methoxy-5,6-dihydrolycopene.—The following directions were adapted from the procedure of Karrer and Takahashi[25] given for the etherification of zeaxanthin. A solution of 50 mg. of 5,6-dihydroxy-5,6-dihydrolycopene in 15 ml. of boiling, dry toluene was mixed with a solution of 25 mg. of potassium in 0.5 ml. of *t*-amyl alcohol (diluted to 1 ml. with boiling dry toluene). The dark brown mixture was kept at 60° for 15 min., cooled to 0°, then, after addition of 2 ml. of methyl iodide, warmed to 60° and kept there for an hour. After dilution with 60 ml. of hexane, the solution was washed alkali-free, dried, and evaporated. The residue was dissolved in 100 ml. of hexane, with the initial aid of a few drops of chloroform. Developing on a 24 × 4.5 cm. column required 0.5 l. of chloroform–hexane 1:5. Photometrically established yield (including *cis* forms),

5 brown-orange
7 orange
15 orange (unchanged starting material)
12 orange
67 yell.-orange (all-*trans*-5-hydroxy-6-methoxy-5,6-dihydrolycopene)
54 yellow (*cis* isomers)

28 mg. (56%); weight of crystals, 12 mg. (24%); thin, nearly rectangular, pinkish-orange plates, m.p. 146–147°; partition ratio, 84:16 in hexane–95% methanol; maxima, 486, *455*, 430 mμ; adsorbs on lime–Celite above 5,6-dimethoxy-5,6-dihydrolycopene but just below lycopene; reaction with acid chloroform, negative.

Lycopene Mono-epoxide.—To 100 mg. of lycopene in 400 ml. of warm abs. ether, was added 100 mg. of monoperphthalic acid in 200 ml. of ether. After standing a week in darkness some lycopene crystals (about 50 mg.) were filtered off. The filtrate was washed with two 100-ml. portions of saturated sodium bicarbonate, then dried, and evaporated. The hexane solution of the residue was developed on a 24 × 4.5 cm. column with 400 ml. of chloroform–hexane 1:5.

21 brown and pale pink zones
5 colorless interzone
94 pink-orange (unchanged-lycopene, ~ 30 mg.)
19 two yellow-orange zones
10 colorless interzone
12 pale orange (lycopene mono-epoxide)

The photometrically estimated yield was as low as 1.5 mg. (1.5%); partition ratio, 99:1 in hexane–95% methanol. The spectrum was similar to that of 5,6-dihydroxy-5,6-dihydrolycopene: maxima, 487, *455*, 428 mμ. The epoxide was adsorbed on lime–Celite slightly above 5,6-dimethoxy-5,6-dihydrolycopene; reaction with acid chloroform, negative.

Hydrolysis.—One mg. of epoxide in 20 ml. of hexane was shaken with 10 ml. of 10% methanolic KOH for 24 hr. The epiphase was washed alkali-free and developed on a 20 × 3.5 cm. column with 400 ml. of chloroform–hexane 3:7. The main, pale orange zone contained 0.2 mg. of a pigment which was shown by spectrum, partition behavior and mixed chromatogram test to be identical with a sample of 5,6-dihydroxy-5,6-dihydrolycopene *ex* lycopene–BF_3.

γ-Carotene. **Formation and Cleavage of the γ-Carotene-BF_3 Complex.**—A total of 250 mg. of γ-carotene was treated in ten portions as follows. To a solution of 25 mg. of pigment in 25 ml. of alcohol-free chloroform (20°) 1 ml. of BF_3-etherate was added with vigorous agitation, and exactly 120 sec. later the then dark blue-green liquid was poured into a

(23) The ash content could not be removed even by washing the benzene solution 30 times with conductivity water. This applies also in other similar instances.

(24) W. Lijinsky and L. Zechmeister, *Arch. Biochem. and Biophys.*, **52**, 358 (1954).

(25) P. Karrer and T. Takahashi, *Helv. Chim. Acta*, **16**, 1163 (1933)

mixture of 10 ml. of saturated sodium bicarbonate solution, 10 ml. of distilled water and 75 ml. of C.P. acetone (red carotenoid color restored). After dilution to 200 ml. with hexane, the solution was allowed to stand until all ten portions were ready. The combined pigment was then transferred to hexane by careful addition of water, the epiphase was washed acetone-free, dried and evaporated; photometrically estimated pigment recovery, 99 mg. of "γ-carotene" (40%). The evaporation residue was dissolved in 100 ml. of hexane (with the initial aid of a few drops of chloroform) and developed on four 45 × 4.5 cm. columns with 500 ml. of hexane–4% acetone each.

92 seven "top zones"
24 pale pink (unchanged γ-carotene, 2 mg.)
12 colorless interzone
280 many yell.-orange zones and (in part) interzones

The combined "top zones" (containing 20% of the initial pigment) were eluted with ethanol, transferred to hexane, washed ethanol-free, dried, and developed for 2.5 hr. on two 45 × 4.5 cm. columns with 1 l. of acetone–hexane 1:9, for each.

28 a tan and a pink zone
17 orange (4-hydroxy-γ-carotene)
20 orange
15 pink
16 yellow
35 nearly colorless interzone
20 orange (4-keto-γ-carotene)
54 nearly colorless interzone
20 yellow
23 nearly colorless interzone
24 yellow

The corresponding zones of the two chromatograms were combined, eluted with acetone–ethanol 1:1, transferred to hexane, washed acetone-free, dried and rechromatographed on 20 × 3.5 cm. columns. Most zones not designated in the above chromatogram represented partially hydrogenated derivatives; some of them were crystallized.[5]

4-Hydroxy-γ-carotene.—The zone in the above chromatogram contained 6 mg. (2.4%) of this compound which yielded, from benzene–methanol, 0.5 mg. of nearly rectangular, deep orange plates (many with jagged ends), m.p. 144–145°; partition ratio, 82:18 in hexane–95% methanol and 92:8 in hexane–90% methanol; maxima at 438, *460*, 491 mμ (Fig. 5). A sharp OH band appeared at 2.78 μ (in CCl_4). On lime–Celite the compound was adsorbed above 4-hydroxy-β-carotene but below lycopene. The reaction with acid chloroform was positive and rapid. The spectrum of the dehydrated product thus formed showed a broad maximum at 467 mμ but no fine structure in the visible region.

Acetylation.—Into a solution of 2 mg. of 4-hydroxy-γ-carotene in 1 drop of anhydrous pyridine 1 drop of acetyl chloride (C.P.) was introduced. After an hour, 10 ml. of methanol and then 10 ml. of hexane were added. The pigment was transferred to hexane and developed with the same solvent on a 18 × 1.8 cm. column. The main zone, located below that of the unchanged hydroxy compound, contained 1.4 mg. of 4-acetoxy-γ-carotene and showed an unchanged spectrum; partition ratio, 95:5 in hexane–95% methanol. The esterification could be reversed by methanolic KOH.

Etherification.—To a solution of 2 mg. of 4-hydroxy-γ-carotene in 5 ml. of abs. ethanol, 3 drops of the acid chloroform reagent was added. After standing for 12 hr. the pigment was transferred to 10 ml. of hexane, dried, and developed on a 18 × 1.8 cm. column with hexane–2% acetone. The top orange zone included unreacted material, while the lower orange zone contained 0.9 mg. of 4-ethoxy-γ-carotene showing the γ-carotene spectrum; partition ratio, 99:1 in hexane–95% methanol. The compound was hydrolyzed to 4-hydroxy-γ-carotene by the method just described for the preparation of the ether, but using a water–acetone mixture 1:4 instead of ethanol.

Oxidation.—One mg. of 4-hydroxy-γ-carotene was dissolved in 5 ml. of reagent grade chloroform and air was bubbled through for 1 hr. The solution was then developed with hexane–5% acetone (column, 18 × 1.8 cm.). The upper zone (orange) contained unreacted substance, and the lower one (pink) 0.1 mg. (10%) of 4-keto-γ-carotene (see below).

4-Keto-γ-carotene.—The indicated zone in the chromatogram described above contained 1.2 mg. (0.5%) of this ketone; dark red, rhomboidal platelets (from benzene–methanol), m.p. 140–142°; partition ratio 89:11 in hexane–95% methanol and 98:2 in hexane–90% methanol; maximum at 468 mμ. On lime–Celite the compound is adsorbed above γ-carotene and 4-keto-β-carotene but below 4-hydroxy-γ-carotene; acid chloroform reaction, negative.

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[CONTRIBUTION FROM THE AUSTRALIAN DEFENCE SCIENTIFIC SERVICE, DEFENCE STANDARDS LABORATORIES, DEPARTMENT OF SUPPLY]

Bond Refractions and the Nature of Phosphorus–Oxygen Bonds

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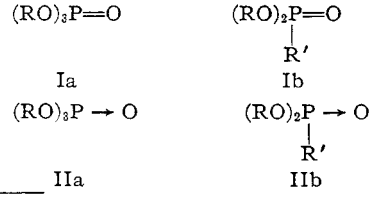
The P–O bond refraction was found to be 3.18 and the P → O bond refraction −1.22 from the experimentally determined molecular refractions of several trialkyl phosphites and phosphates. This supports the formulation of P → O as a coördinate bond. Absence of exaltation and the virtual constancy of the phosphorus–oxygen infrared stretching frequency in diethyl vinylphosphonate and diethyl allylphosphonate, also provide evidence for this view.

In phosphates and phosphonates the phosphorus–oxygen bond commonly represented as a double bond (Ia,Ib) may also be represented as a coördinate bond (IIa,IIb).[1]



(1) The notation X → Y is preferred to X^+-Y^-. The latter implies that the fractional charges on X and Y are equal, which is misleading. The former may be confused with the notation for inductive effect, but this is less likely to be troublesome.

The sulfur–oxygen bond in sulfoxides and sulfones may be similarly represented. In this case the double-bond structure has been supported by Phillips, Hunter and Sutton,[2] and by Cumper and Walker[3] on the basis of dipole moment measurements; by Barnard, Fabian and Koch,[4] and by Amstutz, Hunsberger and Chessick,[5] from a study of hydrogen bonding by infrared spectroscopy;

(2) G. M. Phillips, J. S. Hunter and L. E. Sutton, *J. Chem. Soc.*, 146 (1945).

(3) C. W. N. Cumper and S. Walker, *Trans. Faraday Soc.*, **52**, 193 (1956).

(4) D. Barnard, J. M. Fabian and H. P. Koch, *J. Chem. Soc.*, 2442 (1949).

(5) E. D. Amstutz, I. M. Hunsberger and J. J. Chessick, THIS JOURNAL, **73**, 1220 (1951).