perature. Concentrated sulfuric acid (0.8 cc.) was added and the solution immediately placed under a reflux condenser (Drierite tube). On swirling, much heat was evolved; after ninety minutes, the solution had cooled to room temperature and a small layer of colorless liquid had separated out below the main solution. Anhydrous copper sulfate (20 g.) was now added, the flask stoppered and vigorously shaken, and then replaced under the reflux condenser because of further heat evolution. At intervals, 20 g. and then 40 g. of anhydrous copper sulfate were similarly added and, after the mixture had finally cooled to room temperature, the flask was stoppered, vigorously shaken, and kept overnight at room temperature.

The copper sulfate was now filtered off and the filtrate plus washings treated with anhydrous calcium hydroxide (5 g.) as previously described. The filtrate and washings were combined and freed from acetone by distillation at atmospheric pressure through the column. The acetone-free still-residue was acid to moist blue litmus paper; it was cooled to room temperature (vol., 152 cc.) and shaken with 100 cc. of dilute aqueous sodium bicarbonate solution. The aqueous layer (108.5 cc.) was withdrawn and the upper layer dried with anhydrous sodium sulfate, filtered, and fractionally distilled. After removal of a 4-cc. fraction (containing acetone and moisture) having $\alpha^{22}D$ −1.45° and 6 cc. having $\alpha^{22}D$ −17.15°, *ten* 6-cc. fractions having $\alpha^{22}D$ −18.4 to −18.7° ($n^{25}D$ 1.3910, b. p., 107° at 737 mm.) were obtained, followed by a fraction of $\alpha^{22}D$ −18.28° ($n^{25}D$ 1.3908). For isopropylidene *levo*-2,3-butanediol, Neish and Macdonald[15] found $\alpha^{25}D$ −19.1° or $[\alpha]^{25}D$ −22.1°; $n^{25}D$ 1.3914; b. p., 110° at 760 mm. It gave[15] a "diphasic azeotrope with water, b. p., 86°." They obtained a yield of only 52% of the theoretical.

Distillation of a 50:50 Mixture of Isopropylidene *levo*-2,3-Butanediol plus Isopropylidene *meso*-2,3-Butanediol.—Isopropylidene *levo*-2,3-butanediol (63 cc.) was mixed with 63 cc. of the *meso*-derivative, giving a solution having $\alpha^{24}D$ −9.45°. This was fractionally distilled through the column previously described, yielding two 3-cc. fractions of $\alpha^{23}D$ −9.88° (yellow) and −17.81° (pale yellow; $n^{25}D$ 1.3909), followed by five 3-cc. fractions of $\alpha^{23}D$ −18.16° to −18.11° ($n^{25}D$ 1.3914, b. p., 107° at 735 mm.), and a series of fractions of gradually diminishing optical rotation and increasing refractive index and boiling point. The last fraction collected boiled at 117° at 735 mm. and had $\alpha^{23}D$ −0.12°, $n^{25}D$ 1.4005.

Summary

Separation of *meso*-2,3-butanediol from its optical isomers *via* the isopropylidene derivatives is readily achieved. Owing to the presence of some *levo*-2,3-butanediol together with the *dextro*-isomer in technical grade "*meso*"-2,3-butanediol, only the *meso*-diol derivative can be isolated in pure condition by *distillation* of the mixed isopropylidene derivatives obtained by reaction of the mixed diols with acetone. Some properties of pure isopropylidene *meso*-2,3-butanediol are given.

The preparation of isopropylidene *levo*-2,3-butanediol in practically quantitative yield is described.

The presence of *levo*- or *dextro*- or of D,L-2,3-butanediol in admixture with the *meso*-isomer may be detected and the proportions ascertained.

levo-2,3-Butanediol is a natural concomitant in the bacterial formation of the mixed 2,3-butanediols produced by a normal fermentation of glucose by *Aerobacter aerogenes*.

PITTSBURGH 13, PA. RECEIVED JUNE 18, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY]

Studies on the Structure of ζ-Carotene[1]

BY H. A. NASH,[2] F. W. QUACKENBUSH AND J. W. PORTER

The occurrence, isolation, absorption spectrum and biological inactivity of ζ-carotene have been reported in previous papers.[3,4,5] In the present study information is presented on the structure of this carotene.

Chemical studies on ζ-carotene were preceded by purification as previously described.[3] Removal of a wax-like impurity, which persisted after chromatographic purification, by sodium ethylate saponification did not promote crystallization nor did subsequent molecular distillation. The molecular distillation was carried out in a small still of the pot type at 110° and was designed to remove more-volatile impurities which might be present. After chromatographic removal of the large amount of steroisomers which were formed at this temperature, a solution of ζ-carotene was obtained which showed a specific absorption coefficient at 3560 Å., 2% higher than that previous to molecular distillation. Specific absorption coefficients at 3560 Å. before and after molecular distillation were 54.3 and 55.4, respectively. The failure of these attempts to effect crystallization necessitated further work with non-crystalline material.

Because of the extreme ease of oxidation on exposure to air, ζ-carotene was kept under carbon dioxide or under high vacuum when not dissolved in hexane. Estimates of sample weight were obtained from light absorption values of the ζ-carotene in hexane solution. This method of obtaining sample weights introduced errors of ±2%.

The studies reported in this paper indicate that the most probable formula of ζ-carotene is $C_{40}H_{64}$ and that it possesses an open chain structure similar to lycopene. The typical 40-carbon atom structure of the carotenoids is indicated by the molecular weights, although the experimental values obtained were slightly high, 582, 578 and 543. The polyisoprenic structure is corroborated by the C-methyl determinations. An open chain structure is indicated by the isopropylidene determina-

(1) Journal Paper No. 340 of the Purdue University Agricultural Experiment Station.

(2) Present address: Pitman-Moore Co., Indianapolis, Indiana.

(3) Nash and Zscheile, *Arch. Biochem.*, **7**, 305 (1945).

(4) Porter, Nash, Zscheile and Quackenbush, *ibid.*, **10**, 261 (1946).

(5) Porter and Zscheile, *ibid.*, **10**, 537 (1946).

tion of Kuhn and Roth[6] in which 1.45 moles of acetone were obtained.

Hydrogenation studies and carbon–hydrogen determinations indicate that the molecule contains a total of either nine or ten double bonds. From spectral absorption data it is deduced that only seven double bonds can be present in the conjugated chromophore. This deduction is based on the following considerations: The absorption spectrum shows maxima at 4250, 4000 and 3780 Å. in hexane and at 4520, 4240 and 4020 Å. in carbon disulfide. The work of Zechmeister and Polgár[7] on 5,6-dihydro-α-carotene and 5,6-dihydro-β-carotene, and the work of Karrer and Jucker[8] on epoxy and furan derivatives of α- and β-carotenes indicates that each of the ring double bonds in α- and β-carotene shifts the longest wave length maximum (hexane solution) only about 40 Å. toward the red end of the spectrum. An additional conjugated double bond in the aliphatic chain causes a much greater shift toward the red region. If corrections are made for these less-effective ring double bonds, the longest wave length maximum attributable to the ten conjugated double bonds in the aliphatic chain of γ-carotene becomes 4880 Å. and the corresponding maximum of the nine conjugated double bonds in the aliphatic chain of α- or β-carotene becomes 4700 Å. in hexane solution. As shown in Fig. 1, the relationship of the number of conjugated double bonds in the aliphatic chain to the longest wave length maximum of a carotene in hexane solution is a smooth function. Besides α-, β- and γ-carotene and lycopene, additional points on the curve are dehydrolycopene (fifteen conjugated double bonds[9]), phytofluene (postulated five double bonds[10]) and isoprene. By interpolation on the curve it is seen that a carotene with 8 conjugated double bonds in the aliphatic chain would show its longest wave length maximum at about 4500 Å. and that a carotene with 7 conjugated double bonds in the aliphatic chain would show a corresponding maximum at about 4250 Å. This deduction is supported by the fact that the absorption maximum of aurochrome in petroleum ether solution falls at 4280 Å. Aurochrome is thought to contain seven conjugated double bonds in the aliphatic chain.[8]

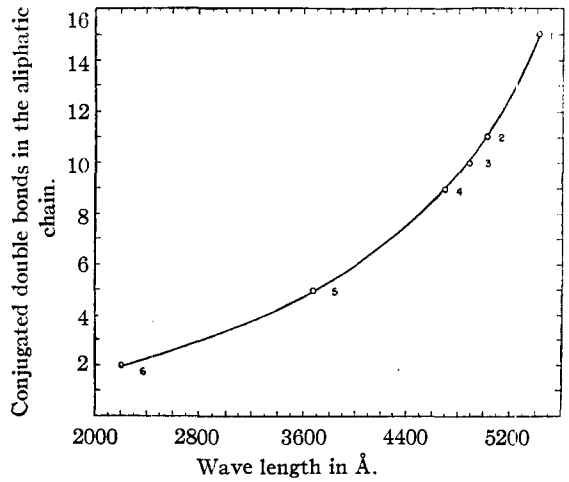
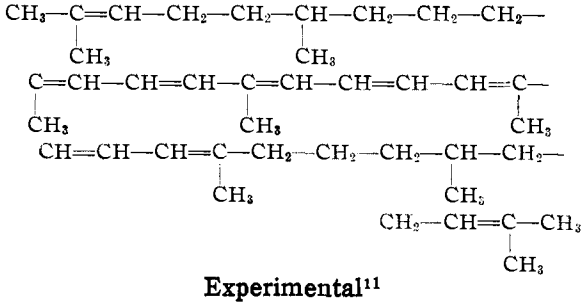


Fig. 1.—Relation of the longest wave length absorption maxima of carotenoids in hexane solution to the number of fully conjugated double bonds: (1) dehydrolycopene, (2) lycopene, (3) γ-carotene, (4) α- and β-carotenes, 5,6-dihydro-α-carotene, (5) phytofluene, (6) isoprene.

The non-identity of ζ-carotene and aurochrome was proved by synthesizing aurochrome by the method of Karrer and Jucker[8] and preparing a mixed chromatogram of ζ-carotene and aurochrome on calcium hydroxide. ζ-Carotene was found to be adsorbed much less tenaciously than aurochrome.

The position of the conjugated chromophore has not been established, but by analogy with lycopene and other carotenoids a central position in the molecule seems most probable. Our failure to observe optical rotation in either a hexane or a carbon disulfide solution tends to support but does not prove this view of molecular symmetry including two rather than three double bonds out of conjugation with the central seven. Oxidation studies to isolate fragments larger than acetone were not successful. Upon oxidation of the limited amount of material available, the steam distillate yielded a very small quantity of substance giving a 2,4-dinitrophenylhydrazone melting above 200° and which was, therefore, thought to be a diketone.

According to the above views, ζ-carotene is 5,6,-7,8,5′,6′,7′,8′-octahydrolycopene ($C_{40}H_{64}$)



Experimental[11]

Materials and Methods.—Alumina (according to Brockmann) was used in the final stages of chromatographic purification. Hexane used as a solvent was a commercial hexane purified by an adsorption method.[12] It was relatively free from unsaturated compounds, since it showed an absorption density of only 0.074 at 2200 Å. (reference solvent—redistilled isoöctane, Röhm and Haas). A Beckman photoelectric spectrophotometer was used for all light absorption measurements.

(6) Kuhn and Roth, *Ber.*, **65**, 1285 (1932).

(7) (a) Zechmeister and Polgár, THIS JOURNAL, **65**, 1522 (1943); (b) Polgár and Zechmeister, *ibid.*, **65**, 1528 (1943).

(8) (a) Karrer and Jucker, *Helv. Chim. Acta*, **28**, 427 (1945); (b) Karrer and Jucker, *ibid.*, **28**, 471 (1945).

(9) Karrer and Rutschmann, *Helv. Chim. Acta*, **28**, 793 (1945).

(10) Zechmeister and Sandoval, THIS JOURNAL, **68**, 197 (1946).

(11) Hydrogenation analyses were performed by Dr. A. J. Haagen-Smit and Dr. G. Oppenheimer, California Institute of Technology, Pasadena, California. Carbon and hydrogen analyses were performed by Mrs. Margaret Murphey of this department.

(12) Porter and Schoeff, unpublished.

Preparation of ζ-Carotene for Analysis.—All ζ-carotene samples except the one used for optical rotation data were prepared by the method outlined in detail in a previous publication.[3] Sample weights were estimated from the light absorption of a hexane solution at 3560 Å. After removal of the solvent under reduced pressure the sample and its container were introduced directly into the carbon–hydrogen train or into the solvents used in other chemical studies.

Molecular Weight.—Molecular weights were determined in camphor by the Rast micromethod. Melting points were observed microscopically with the microscopic hot stage described by Zscheile and White.[13] Using naphthalene, crystalline lycopene and crystalline β-carotene as known substances, the molal depression constant, k, of camphor was found to vary with concentration, a fact previously noted by Meldrum, Saxer and Jones.[14] Since at concentrations great enough to fall on the flat part of the calibration curve carotene solutions in camphor were too concentrated to permit ready observation of melting points, molalities were estimated using 536 as the molecular weight and the k-values corresponding to the estimated molalities were used in calculating the experimental molecular weights.

Analyses.—3.13 mg. of ζ-carotene in 34.8 mg. of camphor (k = 43.0); Δ = 6.65°; 3.13 mg. in 31.1 mg. of camphor (k = 41.2); Δ = 7.17; 3.76 mg. in 35.8 mg. camphor (k = 40.5); Δ = 7.81. Calcd. for $C_{40}H_{64}$: mol. wt., 544. Found: mol. wt., 582, 578, 543.

Carbon and Hydrogen.—Calcd. for $C_{40}H_{64}$: C, 88.15; H, 11.85. Found: C, 87.91, 87.27; H, 11.27, 11.17.

Hydrogenation.—9.98 mg. of substance added 4.04 ml. of hydrogen (0°, 760 mm.). 10.25 mg. added 4.04 ml. 10.98 mg. added 4.11 ml. hydrogen. Found: 9.78, 9.56 and 9.05 double bonds.

C-Methyl Groups.—C-Methyl groups were determined by the method of Kuhn and Roth[15] except that the larger number of distillations recommended by Ginger[16] was used. 13.4 mg. of substance required 17.54 ml. of 0.00865 N barium hydroxide for neutralization of the acetic acid formed. This corresponds, after subtraction of the blank, to 15.80 ml. of base. Found: 5.54 C-methyl groupings.

Isopropylidene Groups.—Isopropylidene groups were determined by the method of Kuhn and Roth[6]: 9.75 mg. of substance in 3 ml. of acetic acid, ozonized for four hours, yielded acetone equivalent to 3.12 ml. of 0.04998 N iodine solution. Expected for 2 isopropylidene groups: 2 moles acetone. Found: 1.45 moles. A control determination on β-carotene, which should yield no acetone, gave 0.22 mole; however, no iodoform precipitate was evident.

Identification of Acetone from Isopropylidene Determination.—Since methyl ketones other than acetone would yield iodoform in the isopropylidene determination, the dibenzylidene derivative of the ketone obtained on ozonolysis was prepared for identification: 55 mg. of substance was ozonized for ten hours in 6 ml. of acetic acid; 40 ml. of water, 32 ml. of 2 N sodium hydroxide and 10 ml. of 1 N potassium permanganate were added and the mixture refluxed for ten minutes; 15 ml. was then steam distilled from the solution and made alkaline with an excess of 0.5 ml. of 10% sodium hydroxide; 5 ml. of ethanol and 3 drops of benzaldehyde were added and the mixture boiled one minute. The precipitate was recrystallized from boiling ethanol: m.p. 112.2° using total immersion thermometer, m.p. of authentic sample of 1,3-dibenzylidene-acetone, 112.3°, mixed m. p. 112.3°.

Addition of 2,4-dinitrophenylhydrazine reagent to 30 ml. of additional distillate gave a very small amount of a precipitate melting above 200°.

Optical Rotation.—Since sodium ethylate might diminish optical activity, chilling to −70° in hexane solution was employed to remove the wax from a sample of ζ-carotene. The wax which precipitated did not evidence optical rotation in either hexane or carbon disulfide solution. 135 mg. of ζ-carotene in 13.8 ml. of hexane: $[\alpha]^{25}D$ 0°; in carbon disulfide: $[\alpha]^{25}D$ 0°. No test of the optical rotation of neo-isomers was attempted.

(13) Zscheile and White, *Ind. Eng. Chem., Anal. Ed.*, **12**, 436 (1940).

(14) Meldrum, Saxer and Jones, THIS JOURNAL, **65**, 2023 (1943).

(15) Kuhn and Roth, *Ber.*, **66**, 1274 (1933).

(16) Ginger, *J. Biol. Chem.*, **156**, 453 (1944).

Summary

Structural studies show that ζ-carotene possesses the typical carotenoid polyisoprenic structure and has an open chain with double bonds in the 1 and 1′ positions as does lycopene. Hydrogenation data show a total of either nine or ten double bonds and light absorption data indicate that seven of these are conjugated. The compound shows no optical activity. From these results it is deduced that the most probable structure of ζ-carotene is 5,6,7,8,5′,6′,7′,8′-octahydrolycopene ($C_{40}H_{64}$).

LAFAYETTE, INDIANA RECEIVED FEBRUARY 28, 1948

[CONTRIBUTION FROM THE DIVISIONS OF ORGANIC CHEMISTRY AND PHARMACOLOGY, THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Streptomycin. VIII. Isolation of Mannosidostreptomycin (Streptomycin B)[1]

BY JOSEF FRIED AND ELWOOD TITUS

In the routine purification of streptomycin concentrates by means of flowing chromatography, essentially as described by Carter, *et al.*,[2] it was observed that the more firmly adsorbed fractions of low bioactivity[3] yielded about two to three times as much maltol per unit of activity on treatment with dilute alkali as the more readily eluted highly active fractions. The isolation from such fractions of a streptomycin-like substance designated Streptomycin B has been reported in a preliminary communication from this laboratory.[4]

The streptomycin concentrates employed in this work were obtained by growing *Streptomyces griseus* in submerged culture in a medium containing soybean meal, glucose and sodium chloride, treating the filtered broth with activated charcoal, and eluting the streptomycin with warm

(1) The nomenclature of streptomycin preparations used in this paper follows the recent suggestion of Prof. S. A. Waksman (*Science*, **107**, 233 (1948)).

(2) H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *J. Biol. Chem.*, **160**, 337 (1945).

(3) All bioassays reported in this paper were carried out with *K. pneumoniae* as the test organism as described by R. Donovick, D. Hamre, F. Kavanagh and G. Rake, *J. Bact.*, **50**, 623 (1945).

(4) J. Fried and E. Titus, *J. Biol. Chem.*, **168**, 391 (1947).