stirred under nitrogen for three hours with 0.5 cc. of dimethyl sulfate and 2 g. of anhydrous potassium carbonate. The mixture became quite dark at the start, but quickly lightened to a pale yellow. At the end of the stirring period the mixture was diluted strongly with water and stirred for fifteen minutes. The pale yellow solid was collected, washed with water, dried and crystallized from glacial acetic acid with the aid of norite, yellow needles mixed with some colorless material, possibly sulfur, 34 mg., m. p. 201–210°. It was recrystallized twice from benzene to give yellow prismatic needles of **3,4-dimethoxyphenanthrenequinoneazine (XIX)**, 20 mg., m. p. 218.5–219°. Its solution in concentrated sulfuric acid is a deep blue-purple color.

Anal. Calcd. for $C_{22}H_{16}N_2O_2$: C, 77.63; H, 4.74. Found: C, 77.38; H, 5.05.

3,4-Dimethoxy-6-chloro-9,10-dioxo-13-cyanomethyl-5,8,9,10,13,14-hexahydrophenanthrene (IV).—A mixture of 4.00 g. of XVII, 28 g. of freshly distilled chloroprene, 0.1 g. of hydroquinone and 40 cc. of purified dioxane was heated for four and one-half days at 85–87° in a pressure bottle. The cooled contents of the bottle were washed out with methanol and the whole mixture was concentrated to dryness by a current of air. The non-volatile dark residue consisted of mixed crystalline material and heavy oil. It was triturated with cold methanol and the insoluble solid material was collected, 1.43 g., m. p. 235–240° with preliminary shrinking and softening from 220°. This solid was washed alternately with bicarbonate and water until the filtrates were no longer red to yield 1.20 g. of olive-green crystalline material, m. p. 259–265°. Recrystallization from methanol with the aid of norite yielded 0.89 g. (16.6%) of nearly colorless small prisms, m. p. 265–267°. A small sample was recrystallized once more for analysis, m. p. 267.5–269°.

Anal. Calcd. for $C_{18}H_{16}O_4NCl$: C, 62.52; H, 4.66; Cl, 10.26. Found: C, 62.29; H, 4.74; Cl, 10.86.

The adduct is very sparingly soluble even in boiling methanol, benzene or ethyl acetate. It is somewhat soluble in boiling dioxane. It is soluble in quite dilute aqueous alkalies to give a pale yellow solution but is difficultly soluble in more concentrated alkalies. Its solution in concentrated sulfuric acid is bright orange-yellow. No trace of silver chloride is produced on boiling the adduct in alcoholic silver nitrate for ten minutes.

3,4-Dimethoxy-6-ethoxy-9,10-dioxo-13-cyanomethyl-5,8,9,10,13,14-hexahydrophenanthrene (V).—A mixture of 4.00 g. of XVII and 12 g. of 2-ethoxybutadiene was heated for two days at 85–87° in a pressure bottle. The resulting viscous brown tar was taken into benzene and separated into bicarbonate soluble, alkali soluble and neutral fractions. The alkali soluble fraction, 0.52 g., m. p. 100–120°, was dissolved in alcohol and on standing yielded 92 mg. of crystalline material, m. p. 210–225°. After several recrystallizations 32 mg. of very small nearly colorless prisms, m. p. 226–228°, with softening and much decomposition from about 220°, was obtained. The material was still not homogeneous.

Anal. Calcd. for $C_{20}H_{21}O_6N$: C, 67.59; H, 5.96; alkoxyl, 30.14. Found: C, 66.20; H, 6.41; alkoxyl, 27.3.

Summary

The synthesis of 5,6-dimethoxy-4-cyanomethyl-1,2-naphthoquinone and its condensation with several conjugated dienes to yield 3,4-dimethoxy-9,10 - dioxo - 13 - cyanomethyl - 5,8,9,10,13,14-hexahydrophenanthrene derivatives are reported.

BRYN MAWR, PENNSYLVANIA RECEIVED AUGUST 16, 1949

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

The Oxidation of β-Carotene

BY N. L. WENDLER,* C. ROSENBLUM AND M. TISHLER†

The high vitamin A activity of β-carotene[1] has often been ascribed[2,3] to the formation of vitamin A in the animal organism through symmetrical fission of the β-carotene molecule at its center position of unsaturation. Experiments designed to duplicate such a degradation *in vitro* by biochemical methods have met with debatable success.[4]

A consideration of the β-carotene structure (I), suggests the attractive hypothesis that the centrally situated 9:10 double bond might well be the most vulnerable position to reagent attack for reasons of its symmetrical and sterically least hindered disposition. Opposed to favorable stereochemical aspects, however, is the supposition that double-bond interaction in an extended system of conjugated double-bonds of this type would be expected to impart maximal single-bond character at the 9:10 position,[5,3] and thus render this center less favorable to electrostatic orientation of the attacking reagent molecule. Furthermore, the large number of controlled oxidation studies[6] on β-carotene which led to the elucidation of its structure, all resulted in cleavages of the β-carotene molecule wherein the fragments isolated were in full possession of an intact 9:10 double bond.[7] Thus, controlled oxidations with chromic acid and lead tetraacetate[8] resulted in initial end-oxidation in the β-ionone ring giving rise to a series of isolable intermediate oxidation products which were further oxidized with accompanying side chain cleavage to β-carotenone-aldehyde (II). Similarly, controlled oxidation with permanganate[9] was

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(1) von Euler, von Euler and Hellstrom, *Biochem. Z.*, **203**, 370 (1928); C. J. Koehn, *Arch. Biochem.*, **17**, 337 (1948).

(2) Woolf and Moore, *Lancet*, **223**, 13 (1932).

(3) Hunter and Williams, *J. Chem. Soc.*, 554 (1945).

(4) Mattson, Mehl and Deuel, *Arch. Biochem.*, **15**, 65 (1947).

(5) Zechmeister, Le Rosen, Polgár and Pauling, THIS JOURNAL, **65**, 1940 (1943).

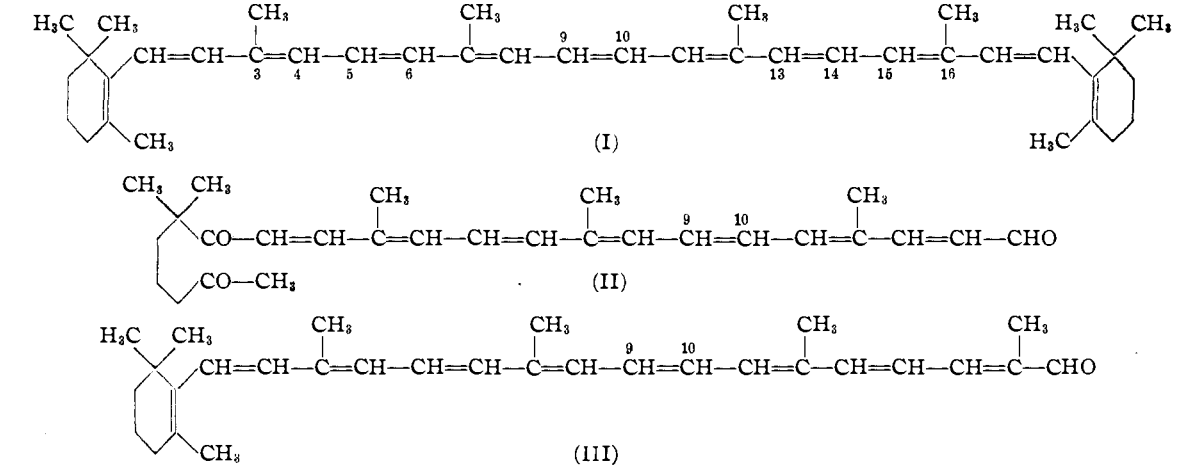
(6) Reviews in "Carotinoide," by Karrer and Jucker, Verlag Birkhäuser, Basel, 1948, p. 133; "The Chemistry of Natural Coloring Matters," by Mayer and Cook, Reinhold Publishing Corp., New York, N. Y., 1943, p. 28.

(7) It should be emphasized that any interpretation with regard to the major course of these and other oxidations must be considered with caution since the severe limitation is imposed, that an interpretation can be defined only in terms of the products identified which, in general, do not represent the major portion of the β-carotene utilized.

(8) Kuhn and Brockmann, *Ber.*, **65**, 894 (1932); *Ann.*, **516**, 95 (1935).

(9) Karrer and Solmssen, *Helv. Chim. Acta*, **20**, 682 (1937); Karrer, Solmssen and Gugelmann, *ibid.*, p. 1020.

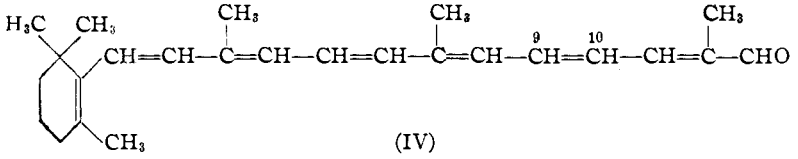
initiated in the β-ionone ring followed by acyclic scission to yield β-apo-2-carotenal (III) and β-apo-4-carotenal (IV) wherein the 9:10 double bond remains preserved. Recent attempts[3] to effect further oxidation of (III) and (IV) to a 9:10 cleavage fragment have proven unavailing. Finally, the action of organic peracids on β-carotene has been demonstrated by Karrer and Jucker[10] to form the epoxides (V) and (VI) as the result of attack in the β-ionone ring, again at the unsaturated centers of least access and greatest substitution.



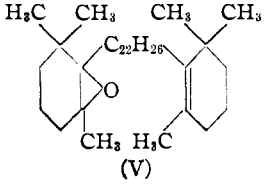
(I)

(II)

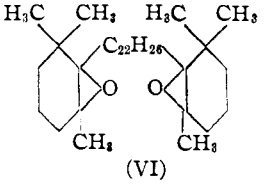
(III)



(IV)



(V)



(VI)

The first observation of an oxidative cleavage at the 9:10 position in β-carotene was reported in 1945 by Hunter and Williams.[3] These workers successfully effected the transformation of β-carotene into vitamin A, although in extremely small amount (0.4–0.5%), by oxidation with hydrogen peroxide in chloroform–acetic acid solution, followed by reduction of the purified oxidation product by means of aluminum isopropylate. More recently, Goss and McFarlane[11] reported that in the presence of osmium tetroxide β-carotene in ether solution is oxidized by hydrogen peroxide to a hypothetical intermediate which disproportionates on subsequent treatment with alkali to give vitamin A directly. The ultraviolet absorption spectrum of the product exhibited maxima at 2830, 3250 and 3700 Å. (cyclohexane). They concluded from the intensity of the 6200 Å. band produced with the antimony trichloride reagent, assuming this color to represent vitamin A only, that the latter had been formed to the extent of 30–40%. In view of the anomalous oxidation sequence proposed by Goss and McFarlane as well as its apparent variance with the results of Hunter and Williams, we undertook a study of the β-carotene–hydrogen peroxide oxidation with the purpose of establishing the course of this reaction through the isolation and identification of the major cleavage fragments formed.

On repetition of the catalyzed oxidation of β-carotene, essentially as described by Goss and McFarlane, we obtained a product exhibiting absorption bands in cyclohexane at 3410 and 3720 Å. with shoulders at ≃3250 and 4025 Å. The absorption spectral characteristics of a typical crude oxidation product (Fig. 1), differed from those of Goss and McFarlane in the important respects that absorption at longer wave lengths was relatively more intense. Thus, the band at 3720 Å. was most pronounced and the 3250 band least distinct. Furthermore, we observed no band in

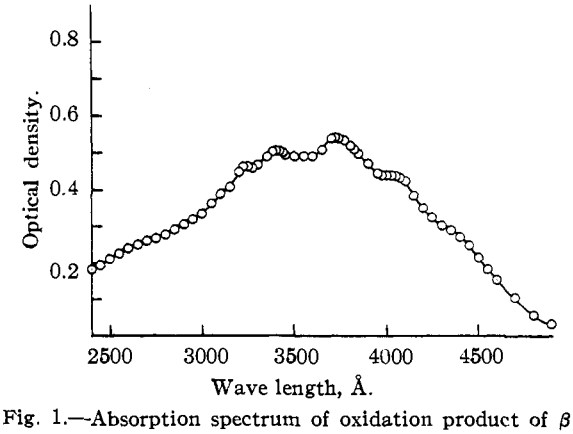


Fig. 1.—Absorption spectrum of oxidation product of β carotene in cyclohexane (250-fold dilution).

(10) Karrer and Jucker, *Helv. Chim. Acta*, **28**, 427 (1945).
(11) Goss and McFarlane, *Science*, **106**, 375 (1947).

the 2800 Å. region. The oxidation product was found to be free of acidic components and could be converted to a crude, mixed-semicarbazone derivative representing approximately the total mass of the oxidation product. This demonstrates, empirically at least, that our oxidation process proceeds almost completely by cleavage of the β-carotene molecule into carbonyl fragments.

The crude oxidation product was examined further by chromatographic resolution using acid-washed alumina as the adsorbate and eluting with various mixtures of benzene and petroleum ether. The product obtained from the first fractions developed an orange color with antimony trichloride reagent and exhibited maximum absorption at 3075 Å. (isoöctane). A crystalline semicarbazone derived from this fraction had a m. p. 194–195° and a $\lambda_{max.}$ 3230 Å., $E^{1\%}_{1\,cm.}$[12] 1370 (chloroform). This substance proved to be β-ionylideneacetaldehyde (VII), previously prepared synthetically by Kuhn and Morris[13] but never before isolated from natural sources. Based on the amount of semicarbazone isolated, β-ionylideneacetaldehyde was found to be formed from β-carotene in yields as high as 30% in some experiments.

The product from the second chromatographic fraction gave a deep blue color with antimony trichloride in chloroform and possessed a single, well-defined band at 3740 Å. measured in isoöctane and 3760 Å. in cyclohexane. This material was found to contain 60–70% vitamin A aldehyde (VIII) formed from β-carotene in yields as high as 30%[14] (Fig. 2). It afforded a crystalline semicarbazone, m. p. 188–190° dec., $\lambda_{max.}$ 3850 Å., $E^{1\%}_{1\,cm.}$ 1540 (chloroform). From a second vitamin A aldehyde preparation there was obtained a semicarbazone, m. p. 199–201° dec., $\lambda_{max.}$ 3850 Å., $E^{1\%}_{1\,cm.}$ 1860 (chloroform) (Fig. 3).

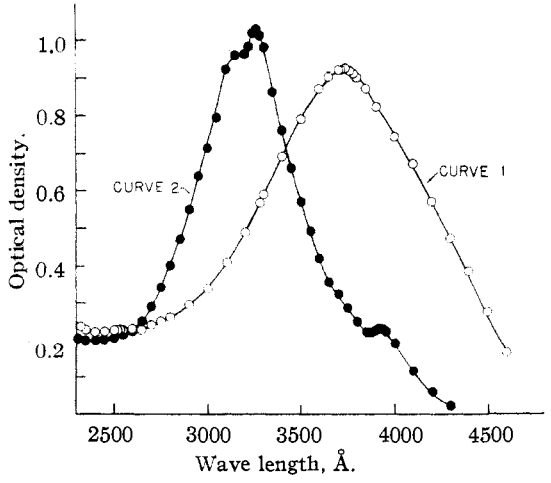


Fig. 2.—Absorption spectra in isoöctane of vitamin A aldehyde (curve 1, dilution 200 ×) and vitamin A alcohol (curve 2, dilution 100 ×) from oxidation of β-carotene.

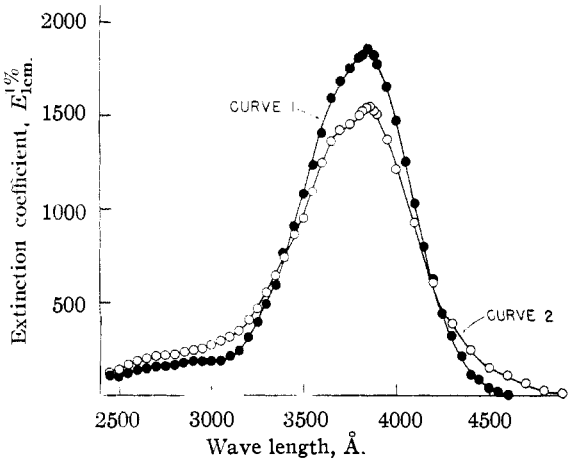


Fig. 3.—Absorption spectra in chloroform of semicarbazones of vitamin A aldehyde from the oxidation of β-carotene: curve 1, product with m. p. 199–201°; curve 2, product with m. p. 188–190°.

Vitamin A aldehyde thus obtained from β-carotene was found to possess a biological activity in the order of 2,800,000 units per gram.[15] It was converted smoothly and in good yield to vitamin A $\lambda_{max.}$ 3260 Å. (isoöctane) by reduction with lithium aluminum hydride (Fig. 2).

From the further chromatographic purification of the β-carotene oxidation product, there was isolated directly and in crystalline form a third substance melting at 168°. This compound formed a semicarbazone derivative decomposing above 250° which unfortunately, for reasons of its extreme insolubility, defied all attempts to purify by recrystallization. This oxidation fragment has been assigned the structure of 2,7-dimethyloctatriendial (IX) on the basis of its analysis and triplet absorption[16] (Fig. 4). This compound was isolated (6–8% from β-carotene) only in those instances where the yield of β-ionylideneacetaldehyde was high and the yield of vitamin A aldehyde correspondingly low. The formation of this dialdehyde would be anticipated, moreover, as a second component in the formation of β-ionylideneacetaldehyde at the expense of vitamin A aldehyde.

We repeated the oxidation conditions of Hunter and Williams,[3] *viz.*, hydrogen peroxide in chloro-

(12) All of our spectral measurements were determined with a Beckmann Model DU quartz spectrophotometer using 1 cm. cuvettes.

(13) Kuhn and Morris, *Ber.*, **70**, 853 (1932).

(14) This yield was calculated on the basis of an $E^{1\%}_{1\,cm.}$ of 1548 at 3730 Å. (cyclohexane), reported for crystalline vitamin A aldehyde by Ball, Goodwin and Morton, *Biochem. J.*, **42**, 516 (1948).

(15) Bioassays were negotiated through the courtesy of Dr. W. L. Sampson of the Merck Institute and were carried out at the Food Research Laboratories, Long Island City, N. Y. A sample containing 56 mg. of vitamin A aldehyde in 116 cc. of Wesson oil showed a vitamin A activity of *ca.* 1340 U. S. P. units/ml.

(16) Bixindialdehyde $(O{=}\overset{H}{C}{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=}CH{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH{=})_2$ exhibits triplet absorption at 4375, 4680 and 5020 Å. (petroleum ether); Kuhn and Grundmann, *Ber.*, **65**, 1880 (1932).

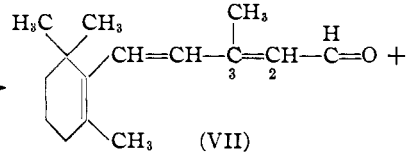
form–acetic acid solution. The oxidation of β-carotene under these conditions was found to be extremely rapid. By employing the technique described above for purification of the oxidation product, we were able to obtain vitamin A aldehyde ($\lambda_{max.}$ 3700–3750 Å.) in about 3–4% yield, thus confirming the original observations of these authors. The vast superiority of the osmium tetroxide–hydrogen peroxide oxidation method can be attributed to the striking catalytic specificity of the osmium tetroxide catalyst, since, in the absence of this catalyst, β-carotene can be recovered essentially unchanged after prolonged contact with hydrogen peroxide in ether solution.

It may therefore be concluded, insofar as the course of the hydrogen peroxide–osmium tetroxide oxidation of β-carotene can be defined by the cleavage products which we were able to isolate that, contrary to previous experience, the oxidation can proceed to a major extent at those unsaturated centers, 5:6 (13:14) and 9:10, which are least substituted and sterically most accessible. Furthermore, aside from the spectroscopic detection of vitamin A aldehyde by Hunter and Williams,[3] none of the products herein reported, *viz.*, β-ionylideneacetaldehyde, vitamin A aldehyde and 2,7-dimethyloctatriendial have heretofore been isolated and characterized from oxidation studies performed on β-carotene.

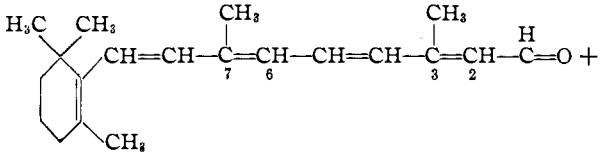
β-Carotene

(I)

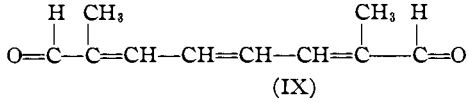
H_2O_2–OsO_4, Ether



(VII)



(VIII)



(IX)

It was mentioned earlier that vitamin A aldehyde was found to give rise to two semicarbazone derivatives. On rare occasions the form was obtained directly which melted at 199–201° dec., and had a $\lambda_{max.}$ 3850 Å., $E^{1\%}_{1\,cm.}$ 1860 measured in chloroform. When measured in isopropyl alcohol this same semicarbazone exhibited doublet absorption of essentially equal intensity at 3680 Å., $E^{1\%}_{1\,cm.}$ 1600 and 3800 Å., $E^{1\%}_{1\,cm.}$ 1660. From the majority of vitamin A aldehyde preparations, however, there was obtained the semicarbazone m. p. 188–190° dec., $\lambda_{max.}$ 3850 Å., $E^{1\%}_{1\,cm.}$ 1540

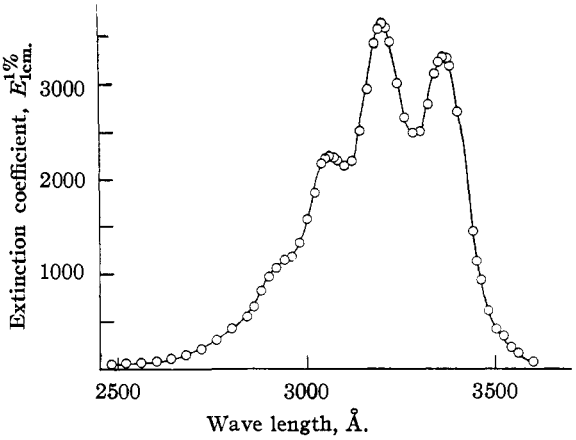
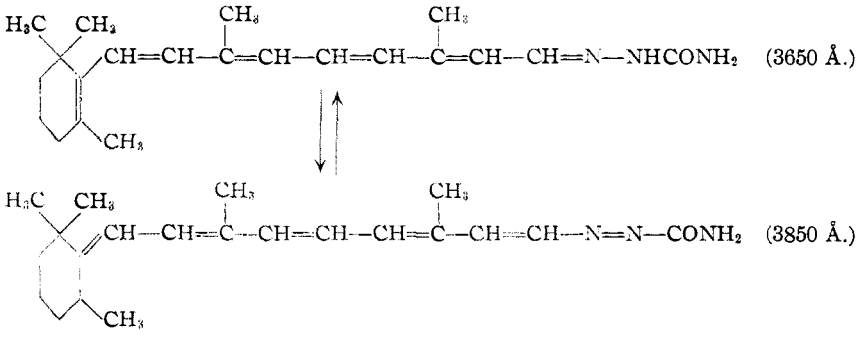


Fig. 4.—Absorption spectrum in isoöctane of 2,7-dimethyloctatrienedial.

(chloroform), showing doublet absorption in isopropyl alcohol at 3650 Å., $E^{1\%}_{1\,cm.}$ 1570 and 3800 Å., $E^{1\%}_{1\,cm.}$ 1650. Both semicarbazone samples analyzed correctly and a mixed melting point of the two modifications gave an intermediate value. The melting point of the lower melting form (188–190° dec.) rose, after two recrystallizations, to the value of the higher melting form (199–201° dec.). In a recent publication by Ball, Goodwin and Morton,[14] an elegant series of experiments is described whereby vitamin A is converted in high yield to vitamin A aldehyde by means of manganese dioxide oxidation in petroleum ether solution. A sample of crystalline vitamin A aldehyde obtained by these authors gave a semicarbazone, m. p. 193–195° (without decomposition), $\lambda_{max.}$ 3850 Å., $E^{1\%}_{1\,cm.}$ 2062 (chloroform). From a second vitamin A aldehyde preparation, stated to be of high purity, there was obtained a second semicarbazone, m. p. 161–164°, $\lambda_{max.}$ 3850 Å., $E^{1\%}_{1\,cm.}$ 1742 (chloroform). The latter after standing in solution in the cold for some time was reported to exhibit a decrease in the 3850 Å. band intensity together with the appearance of a second band of equal intensity at 3650 Å. With the exception of the melting points, the absolute values of which appear at present to be a doubtful criterion for characterization,[17] our results parallel for the most part, those of Ball, Goodwin and Morton who concluded that a mobile interconversion of isomeric forms of vitamin A aldehyde semicarbazone occurs. It would seem further indicated, moreover, that the proportion of their respective contributions to any single modification (mixture) isolated must be dependent to a large extent on the conditions of its preparation. The nature of this isomerism has been provisionally ascribed by Ball, Goodwin and Morton to structural isomerization which they represent as follows

(17) van Dorp and Ahrens [*Nature*, **161**, 189 (1947)] report for the semicarbazone of synthetic vitamin A aldehyde a melting point of 207–209° dec. $\lambda_{max.}$ 3750, log $\epsilon_{max.}$ 4.87 (solvent unstated).



The interesting possibility suggests itself that the isomerism may not be peculiar to the semicarbazone derivative, but may be inherently characteristic of the parent aldehyde itself and as such would involve stereochemical rather than structural isomeric differences. In this regard, it has been demonstrated[18] on the basis of interference calculations that the number of double bonds in a polyenic system which can participate in geometrical isomerism are limited to a few chosen centers. In vitamin A aldehyde (VIII), positions (2,3) and (6,7), and correspondingly in β-ionylideneacetaldehyde (VII) position (2,3) only, should be effective in *cis–trans* isomerism. β-Ionylideneacetaldehyde has been isolated recently from a synthetic preparation in two isomeric modifications[19] with absorption bands at 3150 and 3180 Å., respectively. The 3150 modification gave a single semicarbazone, m. p. 194–195°, $\lambda_{max.}$ 3230 Å., $E^{1\%}_{1\,cm.}$ 1330 whereas the 3180 modification gave a single semicarbazone, m. p. 174–175°, $\lambda_{max.}$ 3175 Å., $E^{1\%}_{1\,cm.}$ 1000 measured in chloroform. Neither semicarbazone, however, exhibited doublet absorption in isopropyl alcohol. Moreover, β-ionylideneacetaldehyde isolated from the oxidation of β-carotene has been found without exception to conform exclusively to the 3150 Å. absorbing modification (semicarbazone m. p. 194–195°) thus establishing a fixed configuration (presumably *trans*-) at position (6,7) in vitamin A aldehyde derived from natural sources.[20] Hence, position (2,3) in the vitamin A aldehyde molecule is the only remaining center at which stereoisomerism would be expected to occur. The fact that β-ionylideneacetaldehyde has been found to exist in two isomeric forms lends strong substantiation to the possible occurrence of similar isomerism in vitamin A aldehyde at the α,β-double bond. The probability of this phenomenon seems further indicated by the high and low absorbing vitamin A aldehyde preparations themselves.

(18) L. Pauling, *Fortschr. Chem. Organ. Naturstoffe*, **3**, 203 (1939).

(19) Wendler, Slates and Tishler, THIS JOURNAL, **71**, 3267 (1949).

(20) The assumption is made that β-carotene (I) remains stereochemically homogenous about positions (3,4) and (15,16) and does not suffer inversion at these centers under the conditions of formation of (VII) and (VIII), *cf.* ref. 5 also L. Zechmeister, *Chem. Rev.*, **34**, 267 (1944).

Experimental

(A) Oxidation of β-Carotene with Hydrogen Peroxide-Osmium Tetroxide.—To a suspension of one gram of β-carotene (General Biochemicals β-carotene labeled 90% β-, 10% α-) in 500 cc. of absolute ether contained in a 1-l. 3-necked flask equipped with a mechanical stirrer and dropping funnel, was added 100 g. of anhydrous sodium sulfate. To this mixture was added dropwise 10 cc. of a 2% aqueous solution of osmium tetroxide,[21] and the reaction mixture agitated until the color had darkened noticeably (*ca.* fifteen minutes) due to osmic ester formation. With vigorous stirring 20 cc. of 30% hydrogen peroxide (superoxol) was then added over a period of five minutes.[22] After the hydrogen peroxide had been added, the reaction mixture was stirred for a time until the oxidation mixture became light-orange in color[23] (*ca.* twenty to twenty-five minutes). At this point, 30 cc. of a 5% aqueous sodium bicarbonate solution was added slowly with cooling. After the effervescence had subsided, the ether solution of the oxidation product was decanted into a separatory funnel and the sodium sulfate washed by decantation with ether. The combined ether washings were washed free of osmic oxides by means of cold 5% aqueous potassium hydroxide solution,[24] and finally washed with water until neutral and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 1 g. of crude oxidation product as a red oil.

Oxidation of 200 mg. of β-carotene by the same procedure yielded 210 mg. of crude product with absorption spectral characteristics shown in Fig. 1. Treatment of this product with 200 mg. each of semicarbazide hydrochloride and sodium acetate in aqueous ethanol, produced an amorphous solid. This material was dissolved in chloroform, washed free of inorganic salts with water, dried over anhydrous sodium sulfate and concentrated to dryness. After thorough extraction with petroleum ether to remove any unreacted organic material followed by drying *in vacuo*, this solid, mixed semicarbazone derivative weighed 220–230 mg.

(B) Chromatographic Resolution of the Oxidation Product.—The oxidation product (1 g.) was dissolved in 200 cc. of low boiling petroleum ether and adsorbed on 100 g. of Merck acid-washed alumina. Fractional elution was effected with mixtures of benzene and petroleum ether. The following fractions were obtained with increasing concentrations of benzene in petroleum ether.

(21) 1 cc. of 2% aqueous osmium tetroxide per 200 mg. of β-carotene was later found to be optimal in the formation of vitamin A aldehyde.

(22) The heterogeneous character of this reaction presents a very serious mechanical handicap. The oxidation was found to be very erratic, the results from any single experiment being difficult to duplicate. After the bulk of this work had been completed, it was discovered that the oxidation could be effectively carried out homogeneously in ether solution by employing proportionate amounts of 90% aqueous hydrogen peroxide and osmium tetroxide in *t*-butyl alcohol to give more reproducible results.

(23) It was found that if the oxidation were interrupted before the color transition had reached the light-orange stage, unreacted β-carotene was invariably recovered from the reaction product. Similarly, on allowing the color transition to exceed the light-orange stage, smaller amounts of the anticipated cleavage products resulted.

(24) We were unable to ascribe any function to the alkali treatment beyond its rôle in removing oxides of osmium. Treatment of the oxidation product with alcoholic potassium hydroxide according to Goss and McFarlane[11] led to the same essential results as when alkali treatment in any form was totally omitted. Omission of an alkaline treatment, however, usually resulted in a darkening of the ether solution due to reduction of osmium tetroxide to lower oxides which were subsequently removed in the chromatographic purification.

(1) **β-Ionylideneacetaldehyde.**—The product from the eluate (*ca.* 5–10% benzene in petroleum ether) giving an orange coloration with antimony trichloride in chloroform solution showed maximum absorption at 3075 Å. (In some experiments the maximum appeared at slightly longer wave lengths 3125–50 Å.). This material on treatment with 500 mg. each of semicarbazide hydrochloride and sodium acetate in aqueous ethanol yielded 300–330 mg. (30% based on β-carotene) of crystalline **β-ionylideneacetaldehyde semicarbazone** which formed glittering yellow plates from methanol, m. p. 194–195°; $\lambda_{max.}$ 3230 Å., $E^{1\%}_{1cm.}$ 1370 (chloroform).

Anal. Calcd. for $C_{16}H_{25}ON_3$: C, 69.82; H, 9.09; N, 15.28. Found: C, 69.56; H, 8.82; N, 15.29.

(2) **Vitamin A Aldehyde.**—The product derived from the eluate (*ca.* 10–20% benzene in petroleum ether) which gave a deep blue color with antimony trichloride in chloroform solution, showed maximum absorption at 3690 Å. This material in a total volume of 100 cc. of petroleum ether, diluted $^1/_{200}$ with isoöctane, possessed an optical density of 0.303 at 3690 Å. Based on an $E^{1\%}_{1cm.}$ of 1548[14] there was approximately 19–20 mg. (9–10% from β-carotene) of vitamin A aldehyde present.

This material yielded a **semicarbazone** as yellow crystals from chloroform–ether, m. p. 199–201° dec., with an absorption spectrum shown in Fig. 3.

Anal. Calcd. for $C_{20}H_{31}ON_3$: C, 73.90; H, 9.09; N, 12.30. Found: C, 73.72; H, 8.67; N, 12.02.

The product obtained from the oxidation of a 100 mg. sample of β-carotene afforded an eluate from the chromatographic purification giving a deep blue color in the antimony trichloride reaction and showing maximum absorption at 3740 Å. (isoöctane) and 3760 Å. measured in cyclohexane (*cf.* Fig. 2). This material in a total volume of 25 cc. of petroleum ether diluted $^1/_{200}$ with isoöctane had an optical density of 0.925; diluted $^1/_{250}$ with cyclohexane gave an optical density 0.769. Calculated on the basis of an $E^{1\%}_{1cm.}$ of 1548 there was approximately 30 mg. of vitamin A aldehyde present or *ca.* 30% conversion from β-carotene.

Semicarbazone.—Yellow crystals from chloroform–ether, m. p. 188–190° dec., absorption spectrum shown in Fig. 3.

Anal. Calcd. for $C_{20}H_{31}ON_3$: C, 73.90; H, 9.09; N, 12.30. Found: C, 73.66; H, 8.82; N, 12.11.

A mixture of this semicarbazone with the one melting at 199–201° dec. melted at 192–195°. After being recrystallized once from methanol and a second time from chloroform–ether this semicarbazone which originally melted at 188–190° dec. now melted at 199–201° dec.

From this same oxidation experiment there was also isolated 5–10 mg. (5–10% based on β-carotene oxidized) of **β-ionylideneacetaldehyde semicarbazone**, m. p. 193–195°.

A number of oxidation experiments were performed employing 200-mg. quantities of β-carotene from which were obtained vitamin A aldehyde samples in yields varying from 16–28% and possessing absorption maxima ranging from 3700 to 3750 Å.

(3) **2,7-Dimethyloctatriendial.**—During the elution process of the oxidation product obtained from 1 g. of of β-carotene, a sharp red zone, marking the upper limit of adsorption of the vitamin A aldehyde moiety gradually progressed downwards as the latter component was eluted. After the complete removal of the vitamin A aldehyde, this zone was desorbed with 50–100% benzene and petroleum ether. Evaporation of the solvents yielded a crystalline substance which gave 20–25 mg. (6–8% from β-carotene) of light orange needles from benzene–petroleum ether, m. p. 168°, $\lambda_{max.}$ 3060 Å., $E^{1\%}_{1cm.}$ 2320, 3200 Å., $E^{1\%}_{1cm.}$ 2620 and 3360 Å., $E^{1\%}_{1cm.}$ 2280 measured in isoöctane (*cf.* Fig. 4).

Anal. Calcd. for $C_{10}H_{12}O_2$: C, 73.17; H, 7.32. Found: C, 73.16; H, 7.59.

The mother liquors from the recrystallization of this compound gave a semicarbazone derivative which decomposed above 250°. For reasons of its extreme insolubility it could not be further purified.

(C) **Oxidation of β-Carotene with Hydrogen Peroxide in Chloroform–Acetic Acid Solution.**—A solution of 100 mg. of β-carotene in 40 cc. of chloroform and 100 cc. of glacial acetic acid was treated dropwise with 2 cc. of 30% hydrogen peroxide while stirring. The color change was immediate, the solution becoming very dark in color. After stirring for two minutes, the reaction product was poured into an equal volume of water (ice) and extracted with ether. The ether extract was washed free of acetic acid with cold aqueous 5% potassium hydroxide solution and then washed with water until neutral, dried over anhydrous sodium sulfate and concentrated to dryness. The residue was chromatographed on 10 g. of acid-washed alumina by the method previously described. Elution with benzene–petroleum ether afforded a fraction giving a blue color with antimony trichloride in chloroform. The product from this fraction dissolved in a total volume of 50 cc. of petroleum ether and diluted $^1/_{100}$ with isoöctane exhibited maximum absorption at 3700–3750 Å. (*d*, 0.117) and at 3980–4000 Å. (*d*, 0.108). Calculated on the basis of an $E^{1\%}_{1cm.}$ 1548 there was ⩽ 3–4% of vitamin A aldehyde present.

Conversion of Vitamin A Aldehyde to Vitamin A.—To an excess (100 mg.) of lithium aluminum hydride dissolved in 50 cc. of anhydrous ether was added dropwise, with stirring, an ether solution containing *ca.* 84 mg. of vitamin A aldehyde. The original orange color of the solution became instantly dissipated and stirring was continued for ten to fifteen minutes. The excess of lithium aluminum hydride was decomposed by dropwise addition of water with cooling. The reaction mixture was decanted into a separatory funnel, the aluminum salts extracted with 10% aqueous potassium hydroxide and the ether solution washed with water until neutral and dried over anhydrous sodium sulfate. One to two drops of a 5% petroleum ether solution of α-tocopherol were added to inhibit oxidation. Evaporation of the solvents left a pale yellow oil. This oil in 100 cc. of petroleum ether, diluted $^1/_{100}$ with isoöctane exhibited maxima at 3260 Å. (*d*, 1.03) and 3920 Å. (*d*, 0.235) shown in Fig. 2. Calculated on the basis of an $E^{1\%}_{1cm.}$ of 1660 at 3260 Å. for pure vitamin A,[25] there must have been formed *ca.* 62 mg. of vitamin A corresponding to a conversion of 70–75% from vitamin A aldehyde.

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Summary

1. The oxidation of β-carotene with hydrogen peroxide–osmium tetroxide has yielded vitamin A aldehyde, β-ionylideneacetaldehyde and 2,7-dimethyloctatriendial.

2. Vitamin A aldehyde obtained from β-carotene was found to possess a biological activity approaching that of vitamin A. It has been converted to vitamin A by reduction with lithium aluminum hydride.

3. It has been found that the oxidation of β-carotene can proceed to a major extent at those unsaturated centers which are least substituted and sterically most accessible.

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