146. *Chemical Conversion of β-Carotene into Vitamin-A.*

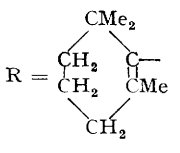
By R. F. Hunter and N. E. Williams.

Claims to have effected conversion of β-carotene into vitamin-A *in vitro* are unsubstantiated by later investigations. The *chemical* conversion has now been accomplished, in small yield, by oxidative fission of the central double bond of β-carotene with hydrogen peroxide to give vitamin-A aldehyde, and subsequent reduction to vitamin-A alcohol.

The mechanism of conversion of β-carotene into vitamin-A which occurs *in vivo* is obscure although the conventional hydrolysis equation $C_{40}H_{56} + 2H_2O = 2C_{20}H_{29}OH$ is given in many text books.

Attempts to reproduce the conversion *in vitro* by the aid of biochemical agents have hitherto furnished indefinite or negative results. Olcott and McCann (*J. Biol. Chem.*, 1931, **94**, 185) claimed to have isolated an enzyme from the liver of rats fed on vitamin-A-free diet, which converted a colloidal solution of carotene into vitamin-A. Pariente and Ralli (*Proc. Soc. Exp. Biol. Med.*, 1932, **29**, 1209) also stated that they detected an increased response to the antimony trichloride reaction after incubating a colloidal solution of carotene with minced dog liver. A re-investigation of Olcott and McCann's experiments by Drummond and MacWalter (*Biochem. J.*, 1933, **27**, 1342) failed to substantiate their claim, and negative results were also obtained when the liver cells were allowed to take up carotene from the circulating blood before the minced preparation was incubated. The more recent claim of Wilson, Ahmad, and Majumdar (*Indian J. Med. Res.*, 1937, **25**, 85) with regard to the conversion in liver autolysates is not supported by spectroscopic evidence.

A conversion by chemical means which suggests itself consists in oxidative fission of the central double bond of β-carotene (I) to give vitamin-A aldehyde (II) and subsequent reduction of this by the Pondorff method to give vitamin-A alcohol (III) (Hawkins and Hunter, J., 1944, 411).



$$\left\{\begin{array}{l} R\cdot[CH{:}CH\cdot CMe{:}CH]_2\cdot CH = CH\cdot[CH{:}CMe\cdot CH{:}CH]_2\cdot R \quad (I.) \\ \downarrow \\ R\cdot[CH{:}CH\cdot CMe{:}CH]_2\cdot CHO + CHO\cdot[CH{:}CMe\cdot CH{:}CH]_2\cdot R \quad (II.) \\ \downarrow \\ R\cdot[CH{:}CH\cdot CMe{:}CH]_2\cdot CH_2\cdot OH + OH\cdot CH_2\cdot[CH{:}CMe\cdot CH{:}CH]_2\cdot R \quad (III.) \end{array}\right.$$

Attempts were made to accomplish the desired oxidation by means of a variety of reagents. A careful repetition of Euler, Karrer, and Solmssen's oxidation of β-carotene with alkaline potassium permanganate (*Helv. Chim. Acta*, 1938, **21**, 211), which furnished the biologically active β-*apo*-2- and β-*apo*-4-carotenals (IV and V), failed to reveal any vitamin-A aldehyde (Devine and Hunter, unpublished results). These *apo*-carotenals differ, however, from vitamin-A aldehyde in possessing a methyl group on the carbon atom adjacent to the aldehyde group.

$$R\cdot[CH{:}CH\cdot CMe{:}CH]_2\cdot CH{:}CH\cdot[CH{:}CMe\cdot CH{:}CH]\cdot CH{:}CMe\cdot CHO \quad (IV.)$$

$$R\cdot[CH{:}CH\cdot CMe{:}CH]_2\cdot CH{:}CH\cdot CH{:}CMe\cdot CHO \quad (V.)$$

Finally, the desired reaction was accomplished, in very small yield, by means of hydrogen peroxide in glacial acetic acid under carefully controlled conditions. Vitamin-A aldehyde was separated from other oxidation products by chromatography, characterised spectroscopically, reduced with aluminium *iso*propoxide, and the product chromatographed. The concentrate of vitamin-A alcohol thus obtained showed an absorption maximum at 6210 A. in the antimony trichloride reaction and was characterised by conversion into " cyclised " vitamin-A by treatment with N/30-alcoholic hydrogen chloride (Hawkins and Hunter, *Biochem. J.*, 1944, **38**, 34). The yield, calculated on the basis of the $E_{1\,cm.}^{1\%}$ value of the " cyclised " vitamin at 3700 A., was 0·4—0·5%.

Owing to the sensitivity of the aldehyde and to the poor yield obtained under optimum conditions, the experiment requires dexterity and speed. Nevertheless, 18 out of 28 experiments furnished positive results.

A biological test on a solution of the vitamin-A alcohol in arachis oil carried out by our Nutrition Laboratory showed growth-promoting activity of the expected order.

Evidence was also obtained of the formation of β-*apo*-5-carotenal, which on reduction furnished the corresponding alcohol showing a band at about 6900 A. in the antimony trichloride reaction. This is distinguishable from vitamin-A_2 (Edisbury, Morton, Simpkins, and Lovern, *Biochem. J.*, 1938, **32**, 118) in that on " cyclisation " it gives a substance showing fine structure in the ultra-violet with absorption maxima displaced some 250 A. into the region of longer wave-length from those of " cyclised " vitamin-A_2. These maxima are almost identical with the product of " cyclisation " of axerophthylidene*iso*propyl alcohol (Hawkins and Hunter, *Biochem. J.*, 1944, **38**, 34), from which the compound differs by only a terminal methyl group. Although this provides further evidence that vitamin-A_2 cannot have the *apo*-5-carotenol structure originally suggested by Gillam, Heilbron, Jones, and Lederer (*Biochem. J.*, 1938, **32**, 405), yet the identity of the ultra-violet absorption maxima of " cyclised " vitamin-A and " cyclised " vitamin-A_2 (Embree and Shantz, *J. Biol. Chem.*, 1940, **132**, 619; Hawkins and Hunter, *loc. cit.*) does not seem to be explicable on the basis of Karrer and Bretscher's open-chain formulæ (*Helv. Chim. Acta*, 1943, **26**, 1758) for vitamin-A_2.

Oxidation of a mixture of β-carotene and ψ-α-carotene gave a result similar to those obtained with β-carotene

itself. Furthermore, there was no substantial difference in the results of oxidation experiments with freshly recrystallised β-carotene and with β-carotene which had undergone slight atmospheric oxidation.

Oxidation of β-*apo*-2- and β-*apo*-4-carotenals with hydrogen peroxide in acetic acid under similar conditions failed to furnish any detectable quantities of vitamin-A aldehyde.

Attempts to increase the yield of vitamin-A aldehyde by variation of conditions (temperature, time of reaction, concentration of the reactants, and use of tocopherol as antioxidant during separation) were unsuccessful. This is explicable on the basis of considerations of resonance in extended conjugated systems, since each double bond loses some of its double-bonded character to the neighbouring single bonds and this effect increases towards the centre of the system (Zechmeister, LeRosen, Schroeder, Polgár, and Pauling, *J. Amer. Chem. Soc.*, 1943, **65**, 1940).

Experimental.

The β-carotene, prepared as described by Devine, Hunter, and Williams (*Biochem. J.*, 1945, **39**, 5), was purified by chromatography and recrystallisation. A value of $E^{1\%}_{1cm.} \leqslant 2{,}200$ at 4630 A. in chloroform was considered satisfactory for this work. Ultra-violet spectroscopic measurements were made in purified *cyclo*hexane. The following experiments are typical.

(i) A solution of β-carotene (100 mg.) in chloroform (40 c.c.) and glacial acetic acid (100 c.c.) was stirred mechanically at 38—40°, in a stream of nitrogen, during the dropwise addition (10 minutes) of 100-vol. hydrogen peroxide (0·08 c.c., equivalent to 3 atoms of oxygen) in glacial acetic acid (20 c.c.). Stirring was continued for a further 10 minutes, and the solution was poured into water and extracted with ether–light petroleum. The united extracts were washed in turn with water, 5% aqueous sodium bicarbonate, and water, dried with anhydrous sodium sulphate, and evaporated under reduced carbon dioxide pressure with the usual precautions (Hunter and Scott, *Biochem. J.*, 1941, **35**, 31). The residue was chromatographed on partly deactivated "Mayfair" alumina under slight carbon dioxide pressure. The chromatogram formed a series of zones: (1) yellow, (2) pink, (3) broad yellow, (4) broad orange pink, (5) bright yellow, (6) very pale yellow, (7) pink, in order of decreasing adsorption. The substances eluted from all these zones were examined with respect to the antimony trichloride reaction. The substance obtained from zone (3) showed absorption maxima at 6700 and 7350 A. in this reaction. The product obtained on Pondorff reduction with aluminium *iso*propoxide showed bands at 6830 and 6200 A. On "cyclisation" with N/30-alcoholic hydrogen chloride at 50° in the usual way, and chromatography on alumina, a typical yellow "cyclised" zone was obtained. This was rechromatographed and two zones were obtained, the lower of which furnished "cyclised" vitamin-A, identified by the characteristic triplet in the ultra-violet region (maxima at 3900, 3700, and 3500 A.), and a maximum at 6200 A. in the antimony trichloride reaction. The upper zone furnished a substance which gave a "cyclised" product having the characteristic triplet displaced to 4150, 3930, and 3700 A., and showing maxima of equal intensity at 6220 and 6850 A. in the antimony trichloride reaction. These ultra-violet maxima are very close indeed to those of the product of "cyclisation" of axerophthylidene*iso*propyl alcohol (Hawkins and Hunter, *Biochem. J.*, 1944, **38**, 36). It is therefore highly probable that this substance is derived from the higher homologue of vitamin-A aldehyde, β-*apo*-5-carotenal. The material eluted from zone (4) showed an intense maximum at 6550 A. in the antimony trichloride reaction, and on reduction with aluminium *iso*propoxide furnished a mixture of alcohols which showed maxima at 6950 and 6200 A. in the same reaction. On "cyclisation" and chromatography, this furnished "cyclised" vitamin-A (maxima at 3900, 3700, and 3500 A., and at 6200 A. in the antimony trichloride reaction). Evidence was also obtained of the presence of "cyclised" β-*apo*-5-carotenol.

(ii) A similar oxidation experiment was carried out at 48—50° and the product was chromatographed as before. The four intermediate zones which showed bands at 6570 and 6950 A. in the antimony trichloride reaction were eluted together, and the product was reduced, "cyclised," and chromatographed. The lower pale yellow zone on being rechromatographed gave material which showed absorption bands at 4120, 3900, 3700, and 3500 A. in the ultra-violet, and maxima at 6250 and 7000 A. in the antimony trichloride reaction. The solution of this material in *cyclo*hexane (25 c.c.) showed a log I_0/I value of 1·03 in a 0·2-cm. cell at 3700 A., corresponding to a yield of 0·35% of vitamin-A on the basis of an $E^{1\%}_{1cm.}$ value of 3650 for pure "cyclised" vitamin-A. On being again chromatographed this fraction was resolved into two zones: the lower showed ultra-violet maxima at 3900, 3700, and 3500 A. and a band at 6220 A. in the antimony trichloride reaction, and the upper zone showed maxima at 4150, 3900, and 3700 A. and a band at 7000 A. by the two methods (compare the separation of "cyclised" vitamin-A and "cyclised" vitamin-A_2; Embree and Shantz, *loc. cit.*; Hawkins and Hunter, *loc. cit.*).

(iii) An experiment similar to (i) was carried out in which β-carotene (50 mg.) was oxidised with hydrogen peroxide (equiv. to 3 atoms of O, added dropwise during 4 minutes) at 50°. After being stirred for a further minute, the reaction mixture was poured into dilute aqueous ferrous sulphate and extracted with light petroleum. The "cyclised" vitamin separated by chromatography showed only vague absorption in the ultra-violet region. The experiment was repeated with a similar result. The "cyclised" products were therefore combined and rechromatographed on a longer column of "Mayfair" alumina; a small fraction was obtained which showed vague absorption bands at 3900, 3700, and 3500 A. The amount of vitamin isolable under these conditions was almost negligible.

(iv) An experiment similar to (iii) was carried out in which β-carotene (50 mg.) was treated with hydrogen peroxide (equiv. to 3 atoms of oxygen) at 25° during 10 minutes. Stirring was continued for a further 10 minutes. The alcohol obtained on Pondorff reduction showed bands at 6220 and 6930 A. in the antimony trichloride reaction. The "cyclised" vitamin (yield 0·5%) showed absorption maxima at 4150, 3900, 3700, and 3500 A. in the ultra-violet, and the same bands with antimony trichloride as before.

(v) A similar experiment in which β-carotene (100 mg.) was oxidised at 15° furnished "cyclised" vitamin (yield 0·45%) which showed maxima at 3900, 3700, and 3500 A.; some 48 mg. of unchanged carotene were recovered.

(vi) An experiment in which 50 mg. of β-carotene were oxidised with hydrogen peroxide (equiv. to 6 atoms of oxygen) at 38—40° also furnished a concentrate which showed bands at 6220 and 6900 A. in the antimony trichloride reaction. The "cyclisation" product showed maxima at 4120, 3900, 3680, and 3500 A.

(vii) A ψ-α-carotene concentrate (*ca.* 30% of ψ-α-carotene) was prepared by heating a solution of β-carotene in benzene–glacial acetic acid (1 : 2) and subsequent chromatography. A solution of the carotene (150 mg.) in chloroform (60 c.c.) and glacial acetic acid (150 c.c.) was oxidised with hydrogen peroxide at 38—40° in the usual way. The "cyclised" vitamin showed bands at 4150, 3900, 3700, and 3500 A. in the ultra-violet, and maxima at 6210 and 6950 A. in the antimony trichloride reaction; yield 0·47%.

Oxidation of β-apo-2-*Carotenal.*—A solution of β-*apo*-2-carotenal (m. p. 140°) (20 mg.) in chloroform (10 c.c.) and glacial acetic acid (30 c.c.) was treated with hydrogen peroxide (equiv. to 3 atoms of oxygen) at 40° as in previous experiments. The product was reduced with aluminium *iso*propoxide and thereafter treated with N/30-alcoholic hydrogen chloride in the usual way. On chromatography, four zones were obtained: (1) bright yellow, (2) deep salmon pink, (3) orange

red, and (4) orange, in order of increasing adsorption, none of which furnished material showing fine structure in the ultra-violet region.

Oxidation of β-apo-4-*Carotenal.*—A solution of β-*apo*-4-carotenal (Euler, Karrer, and Solmssen, *loc. cit.*) (50 mg.) was oxidised at 40°, and the product reduced and treated as in the previous case. The chromatogram formed a series of six zones, none of which showed fine structure in the ultra-violet region.

Biological Test.—One-third of a fresh concentrate of vitamin-A alcohol, prepared by Pondorff reduction of the aldehyde obtained by oxidation of 200 mg. of β-carotene, was "cyclised," and the log I_0/I value at 3700 A. determined in the usual way. The remaining two-thirds was dissolved in refined deodorised arachis oil containing tocopherol (22 mg. per g. of oil), to furnish a solution with a nominal potency of 240 International units per g. which was used as the dosing solution for growth tests on rats on the usual basal diet (Gridgeman, *Biochem. J.*, 1943, **37**, 127). The test, the essential feature of which consisted of 10 litter-mate growth comparisons, indicated that the potency was at least of the expected order. The animals receiving no supplement showed no growth during the 3-week test period (5 died), whilst those receiving the test material showed an average growth of 54 g.

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