CXXXVIII. THE ISOMERISATION OF CAROTENE BY MEANS OF ANTIMONY TRICHLORIDE.

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(Received May 17th, 1932.)

THE stable blue solution (λ max. 583–590 $m\mu$) obtained by the action of antimony trichloride on carotene in chloroform has been poured into water and the organic matter recovered free from antimony. The product is different from, but isomeric with, carotene (compare "regeneration" experiments on vitamin A [Edisbury *et al.*, 1932]).

The carotene used (m.p. 180°) was a commercial sample recrystallised from pyridine. Purified in this way carotene exhibits maxima in the spectral absorption curve (solvent, chloroform) at 495, 462, 436, 348 and 280 $m\mu$. The absorption attains its highest intensity at 462 $m\mu$, at which point the molecular extinction coefficient is 102,000 ($E_{1\text{ cm.}}^{1\%}$, 1900), in agreement with the results obtained previously for the purest carotene [*cf.* Dulière, Morton and Drummond, 1929]. The intensity of absorption at the maximum (585 $m\mu$) in the colour test with antimony trichloride can be expressed as $E_{1\text{ cm.}}^{1\%}$, 585 $m\mu$ = 420.

The method adopted in the "regeneration" experiments was as follows. Carotene (1 g.) was dissolved in chloroform (100 cc.) and treated with a saturated solution of antimony trichloride in chloroform (140 cc.). The mixture was allowed to stand for two minutes and was then poured into water, whereupon antimony oxychloride was precipitated and the original colour of the carotene returned. The oxychloride was dissolved in the minimum quantity of concentrated hydrochloric acid and the mixture left to stand, when the chloroform layer gradually separated. The aqueous portion was shaken with chloroform to recover every trace of extractable material, and the united chloroform extracts were repeatedly washed with water till free from all traces of antimony, and finally dried over sodium sulphate. After removal of solvent by evaporation in a current of nitrogen, a red crystalline residue similar to carotene in appearance was obtained.

This material was then boiled with pentane (200 cc.) and the insoluble residue (fraction A) removed by filtration. The filtrate was concentrated to half-bulk when a further crop of crystals, m.p. 170°, was deposited (fraction B), while complete evaporation of the solvent gave a crystalline residue, m.p. 140° (unsharp) (fraction C). Recrystallisation of A from pyridine raised the m.p. to 185° which was not altered by further purification.

All three fractions of the regenerated product when dissolved in chloroform gave solutions slightly redder in colour than those obtained with carotene at the same dilution. The absorption spectrum in the visible region was characterised in each case by a group of maxima similar to that shown by carotene, but displaced in the direction of the longer wave-lengths. The absorption in the ultra-violet was totally different from that of carotene (see Fig. 1).

Material	M.P.	Wave-length maxima in $m\mu$						
		509	482	454	425	389	367	317
Gross "regenerated"	—	509	482	454	425	389	367	317
Fraction A	185°	512	483	453	(426)	389	—	316
Fraction B	170°	511	480	450	423	389	367	316.5
Fraction C	140°	510	480	450	(423)	—	—	317
Carotene	180°	497	463	436	—	—	350	280

It will thus be seen that the new material is unmistakeably different from carotene in its optical properties. It exhibits a larger number of narrow absorption bands, and in the visible, where the absorption curve is qualitatively similar to that of carotene, the wave-length displacement is very definite. All three fractions exhibit the same maxima and cannot be differentiated qualitatively.

The regenerated product gives a blue solution with the antimony trichloride reagent exhibiting a maximum at 583–587 $m\mu$, practically identical with that of ordinary carotene. The intensity, however, with the sample of highest m.p. did not reach that obtained with the purest carotene recrystallised from pyridine.

Material	Colour test	Visible absorption	Ultra-violet absorption
	$E_{1\text{ cm.}}^{1\%}$ 585 $m\mu$	$E_{1\text{ cm.}}^{1\%}$ 462(480) $m\mu$	$E_{1\text{ cm.}}^{1\%}$ 317 $m\mu$
Carotene	420	1900	—
Regenerated material:			
Fraction A	370	1800	430
Fraction B	365	1800	435
Fraction C	340	1370	330

Without more elaborate purification on a larger scale it cannot be concluded that the intensities of the absorption bands shown by the regenerated product are much different from those shown by carotene itself.

The three main fractions of the regenerated pigment were examined for growth-promoting activity by tests on young rats, the growth of which had been inhibited by feeding on a diet devoid of vitamin A. The basal diet was compounded of

Purified caseinogen	...	20
Rice starch	...	80
Salt mixture	...	5
Dried yeast	...	5

Vitamin D was supplied by the administration of 0.001 mg. irradiated ergosterol twice a week. This, as well as the recovered pigment, was given in the form of a solution in ethyl laurate. The solutions were prepared twice a week and stored in the dark at low temperature. Only one preparation showed any sign of growth-promoting power, namely, that with the highest melting-point (185°). When administered in amounts which in the case of carotene bring about a good resumption of growth, there was an indifferent response (see Fig. 2). The results of tests with larger doses suggested that the material was mainly composed of an inactive substance but that a small amount of an active component, possibly unchanged carotene, was also present.

Fig. 1. Spectral absorption curves:

---- Carotene (recryst. pyridine).
Calculated to 0.00078 % (1 cm.) CHCl_3 .
— Regenerated product.
Calculated to 0.00075 % (1 cm.) CHCl_3 .

Fig. 2. Biological tests on carotene before and after treatment with antimony trichloride.

The preparations with lower melting-points were devoid of growth-promoting power.

Comparison of the regenerated product with the different varieties of "carotene" studied by Kuhn and Brockmann [1931], and Kuhn and Lederer [1931; 1932] reveals that it is probably identical with *isocarotene*, prepared by the action of either metallic mercury or sodium thiosulphate upon carotene tetraiodide.

	M.P.	Absorption maxima in CS_2 , $m\mu$				Growth- promoting activity
α -Carotene	$174-175^{\circ}$	511	478	448	—	good
β -Carotene	$180-181^{\circ}$	520	485	454	—	good
<i>iso</i> Carotene	$185-186^{\circ}$	543	503	472	—	nil
Regenerated product	185°	540	505	478	445	nil

SUMMARY.

1. When the blue solution obtained by mixing antimony trichloride and carotene in chloroform is poured into water, the original colour of the carotene reappears in the chloroform layer from which red crystals can be isolated.

2. The absorption spectrum of the recovered product has been determined in chloroform and carbon disulphide. Both in the visible and ultra-violet regions it is quite different from that of carotene.

3. The properties of the substance agree with those of *isocarotene*.

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