CCI. THE ISOMERIZATION OF CAROTENES BY CHROMATOGRAPHIC ADSORPTION

II. *NEO*- α -CAROTENE

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PREVIOUS investigations have shown that when pure β -carotene is adsorbed repeatedly on alumina it changes into a pigment having the same absorption spectrum as α -carotene but differing in other properties. After isolation the new pigment was named *pseudo*- α -carotene [Gillam & El Ridi, 1935; 1936].

Preliminary experiments with α -carotene have shown that this compound can also be changed by chromatographic adsorption into yet another pigment differing markedly, in the absorption spectra of its solutions, from the other known carotenes. This pigment, which we have provisionally called *neocarotene* [Gillam & El Ridi, 1936], exhibits absorption maxima at 501 and 471 $m\mu$ as compared with 508 and 477 $m\mu$ for the parent α -carotene (solvent, carbon disulphide). The solution, as judged by its absorption spectrum, is quite stable if kept from contact with oxygen; on crystallization however, it has been found that the crystals which separate are quite different in spectral absorption from the mother liquor, having in fact undergone change into a compound having absorption maxima identical with those of α -carotene. Subsequent experiments have shown that the pigment formed is neither α -carotene nor *pseudo*- α -carotene but a new carotenoid for which the name *neo*- α -carotene is proposed. The present paper describes the preparation and properties of this compound, whilst the isolation and properties of *neocarotene* itself, when completed, will form the basis of a later communication.

Preparation of neo- α -carotene

300 mg. of α -carotene (M.P. 179°; ex palm oil) were adsorbed on a 40 $\times$ 3 cm. column of alumina (Brockmann's "special, for adsorption", diluted 1 : 1 with ordinary alumina) and washed down the column with several litres of light petroleum (60–80° B.P.), plus a little pure benzene.¹

The pigment was adsorbed in a single zone only, but after elution and re-adsorption a good separation into two zones was obtained. The pigment constituting the lower zone (*neocarotene*, absorption maxima in CS₂, 501 and 471 $m\mu$) was washed out of the column and put aside whilst that in the upper zone was eluted and re-adsorbed. As before the two zones appeared and the

¹ It is interesting to note that in contrast to the parallel conversion of β -carotene into *pseudo*- α -carotene the conversion of α -carotene into *neocarotene* occurs as easily on magnesium oxide as it does on alumina [cf. Gillam & El Ridi, 1936].

pigment in the lower zone was again separated and added to the first. In a particular series of adsorptions carried out in this way 300 mg. of α -carotene yielded 90–100 mg. of *neocarotene* in solution (estimated colorimetrically), much of the original pigment being left, apparently unchanged.

The solutions of the eluted pigment from the bulked lower zones were concentrated and allowed to crystallize from the petroleum (with or without the addition of alcohol), three separate crops of crystals being collected. The changes in properties which occur during preparation and crystallization are shown in Table I.

Table I. *Preparation of neocarotene and neo- α -carotene*

	α -carotene max. 477, 447 m μ ↓ adsorbed on alumina (from benzene-petroleum) ↓ <i>neocarotene</i> max. 470, 441 m μ			
	Crystallization			
	↓	↓	↓	↓
	First crop (recryst.)	Second crop (recryst.)	Third crop (recryst.)	Mother liquor from each crop
Absorption maxima:				
Petroleum (60–80° B.P.)	476, 447 m μ	476, 446.5 m μ	477, 447 m μ	470, 441 m μ
Carbon disulphide	509, 476.5 m μ	509, 476 m μ	509, 477 m μ	501, 469 m μ
M.P.	170–172°	170–172°	171–172°	—
Rotation $[\alpha]_{\text{Cd}}$	+220°	+225°	—	—
Analyses:				
C %	89.5	89.33	—	—
H %	10.54	10.60	—	—
Antimony trichloride test:				
Colour	Blue	Blue	Blue	—
Absorption bands	590, 492 m μ	591, 492 m μ	590, 492 m μ	—
Pigment present	← neo- α -carotene →			<i>neocarotene</i>

Properties of neo- α -carotene

The pigment after recrystallization from any of the usual organic solvents is a red glistening solid similar in appearance to the other known carotenes but differing in properties as shown in Table II.

The crude material as first obtained melted at 166°, but on recrystallization this rose to 170–172°. Attempts to raise the melting-point of this and other samples by crystallization from alcohol, light petroleum, hexane, benzene, ether and various mixtures of these solvents failed, and the best value must therefore be taken as 172° (uncorrected). Micro-analyses (for which we are indebted to Mr W. F. Boston of Manchester University) on recrystallized material, gave the following results:

	C %	H %
First crop	89.5	10.54
Second crop	89.33	10.60
Independent preparation	89.35	10.25
Mean values	89.39	10.46
C ₄₀ H ₅₆ requires	89.55	10.45

In solution, the pigment exhibited absorption maxima identical in location with those of α -carotene. Three bands appeared to be present in all cases, but

the short wave band was not locatable with accuracy on a Hilger-Nutting visual spectrophotometer. The observed values were as follows:

Carbon disulphide	509, 476.5 m μ	Ethyl alcohol	476, 447 m μ
Chloroform	486, 455 m μ	Ether	476.5, 446 m μ
Benzene	487, 457 m μ	Petroleum	476, 447 m μ

The optical rotation was determined in filtered white light of wave-lengths near 6438 Å. using a 10 cm. tube and concentrations of the order of 0.1%. Values of $[\alpha]_{\text{cd}}$ in benzene for separate samples of the pigment were as follows:

220, 225, 211, 225; Mean $[\alpha]_{\text{cd}}$ (benzene) = +220°.

When subjected to chromatographic adsorption on alumina *neo*- α -carotene gives three zones of pigment having colours and absorption spectra as follows:

	Colour of zone	Absorption bands (petroleum)	Pigment ?
Upper zone	Pink	480, 451 m μ	β -carotene
Middle zone	Orange	477, 447 m μ	<i>neo</i> - α -carotene
Lower zone	Yellow	471, 442 m μ	neocarotene

Hydrogenation of *neo*- α -carotene according to the micro-method of Jackson & Jones [1936] gave a value of 10.4 for the number of ethylene linkages. This, together with the facts that the parent α -carotene has 11 double bonds, and β -carotene on microhydrogenation often only gives values of 10 for its 11 ethylene linkages [cf. Smith, 1932], can be taken to indicate the presence of a total of 11 double bonds.

Biological tests for vitamin A activity (carried out at the National Institute for Research in Dairying).

(1) *neo*- α -Carotene. The pigment was fed in coconut oil to a group of 8 rats (5 ♂♂ + 3 ♀♀), previously depleted of vitamin A, at a nominal level of 3 γ per rat per day for 5 weeks. The colour of the solution was determined as 1670 Moore [1930] yellow units per mg. per ml. whereas the pure pigment, by comparison with α - and β -carotenes, should be 2200 Moore Y.U. per mg. per ml. The corrected level was therefore 2.27 γ . This produced an average gain in weight of 14.4 g. (over 5 weeks), this and the subsequent gains being averages of sex averages. The pigment was also fed to a group of 4 ♂♂ and 3 ♀♀ at a nominal level of 15 γ and gave an average gain of 29 g. in 3 weeks. As the growth was so rapid this experiment was discontinued. β -Carotene was fed alongside, for comparison, to 7 rats (4 ♀♀ + 3 ♂♂) at a level of 1.75 γ daily. The solution had a colour value of 2000 Moore Y.U. per mg. per ml. making the actual level 1.6 γ . The average gain in weight per rat over 5 weeks was 14.5 g., i.e. almost identical with the *neo*- α -carotene value at the same level. It therefore follows that 2.27 γ of *neo*- α -carotene are equivalent to 1.6 γ of pure β -carotene, i.e. *neo*- α -carotene has about 0.7 of the potency of β -carotene.

In addition to the normal feeding tests two similar vitamin A-depleted rats were given a total of 4 mg. of the *neo*- α -carotene over 4 days and on the 5th day the rats were killed and the livers removed. It was found that an alcoholic extract of the liver unsaponifiable matter exhibited an absorption maximum at 328 m μ , whilst a chloroform extract gave a blue colour with antimony trichloride. The liver of one rat contained 210 γ of vitamin A whilst that of the other contained 100 γ (basis $E_{1\text{cm}}^{1\%}$. 328 m μ = 1600 for pure vitamin A).

(2) *Pseudo*- α -carotene. This pigment was fed in coconut oil to a group of only 4 rats. They were given the carotene twice weekly for 5 weeks at the rate of 1.75 γ per rat daily. The solution of the pigment had a yellow value of 2200

Moore Y.U. per mg. per ml., i.e. it can be taken as pure pigment. The average gain in weight of the rats was 9.5 g. over 3 weeks and 15.8 g. over 5 weeks compared with 6.5 and 10.0 g. respectively for pure β -carotene fed at the same level under strictly comparable conditions. Thus, although the number of rats used was small there is no doubt that *pseudo*- α -carotene is at least as potent as pure β -carotene.

This finding is in agreement with a previous biological test on the pigment, carried out for us at the Glaxo Laboratories, through the kindly co-operation of Mr A. L. Bacharach [cf. Gillam & El Ridi, 1936].

An oil solution of *pseudo*- α -carotene was fed to two groups of rats at levels corresponding with daily doses of 2 and 4 γ per animal per day; a third group was given 2.5 I.U., as a dilution of the International Standard Preparation of Vitamin A, that is, 1.5 γ of pure β -carotene, per animal per day. The numbers of animals in the three groups were, respectively, 6 ♂♂ and 5 ♀♀, 3 ♂♂ and 4 ♀♀, 3 ♂♂ and 4 ♀♀; the weighted means for the average weight increase of each group after 35 days were 44, 56 and 27 g. per animal, respectively.

These tests were not sufficiently extensive to justify basing upon them any quantitative comparison of biological activities; they do, however, point clearly to the conclusion that the vitamin A potencies of *pseudo*- α -carotene and β -carotene are of the same order.

(3) *Neocarotene*. This pigment has so far not been isolated in the solid state, for when allowed to crystallize it changes into *neo*- α -carotene (*vide supra*). As it was considered possible that it might have no vitamin A activity an attempt was made to test it biologically by transferring its solution in ether to coconut oil without allowing it to crystallize. In this way a solution having a yellow value of 2200 Moore Y.U. per mg. per ml. was made up and fed to a group of 4 rats, previously depleted of vitamin A reserves, at a level of 17.5 γ per rat per day. The rats grew, the average rates being 20.8 g. per rat in 3 weeks and 30.8 g. in 5 weeks. Similar rats fed with pure β -carotene at only 1.75 γ per rat daily gave, under comparable conditions, weight increases of 6.5 and 10.0 g. respectively.

It is thus only possible to say that *neocarotene* is definitely biologically active and further that 17.5 γ of *neocarotene* are considerably more effective than 1.75 γ of pure β -carotene, but what is the precise relation existing between the activities of *neocarotene* and β -carotene cannot be decided from the present data.

A single rat previously depleted of vitamin A was also fed a heavier dose of the *neocarotene*, i.e. a total of 3.1 mg. over 5 days. The rat was killed on the 5th day and its liver examined. It was found that on saponification of the liver the extract exhibited the ultraviolet absorption at 328 $m\mu$ characteristic of vitamin A and that the whole liver contained vitamin A equal to 466 B.U. [Moore, 1930].

There is thus no doubt that *neocarotene* is vitamin A-active but there is the possibility that this activity is due to the *neocarotene* having been changed in the body of the rat into the vitamin A-active *neo*- α -carotene. This change has been shown to occur very easily under other conditions.

The properties described above lead to the following conclusions. *neo*- α -Carotene has an ultimate composition corresponding to $C_{40}H_{56}$ and contains 11 ethylene linkages. The absorption spectrum of the pigment, together with its carotenoid nature and the fact that it supports growth in rats on an otherwise vitamin A-deficient diet, would suggest a structure containing a β -ionone ring and 9 ethylene linkages conjugated with it. The remaining double bond would

seem to be isolated as in the case of α -carotene, the structure of which has been shown to be as follows [Karrer *et al.* 1933]:

We therefore have now three isomeric α -carotenes, i.e. natural α -carotene, *pseudo*- α -carotene, and *neo*- α -carotene. Their similarities and differences are summarized in Table II.

Table II. Comparison of the isomeric α -carotenes

	α -Carotene	<i>pseudo</i> - α -Carotene	<i>neo</i> - α -Carotene
Prepared from	Palm oil	β -Carotene	α -Carotene
Formula	$C_{40}H_{56}$	$C_{40}H_{56}$	$C_{40}H_{56}$
Absorption maxima	477, 447 m μ	477, 447 m μ	477, 447 m μ
M.P.	183.5°	166°	170–72°
Rotation $[\alpha]_{D}$	+377°	Nil	+220°
Adsorption on alumina gives zones of pigment with absorption spectra corresponding to	α -Carotene + <i>neocarotene</i>	β -Carotene + <i>pseudo</i> - α -carotene	β -Carotene + <i>neo</i> - α -carotene + <i>neocarotene</i>

The absorption spectrum, optical rotation and adsorption properties of *neo*- α -carotene (Table II), might be simulated by a mixture of β -carotene and *pseudo*- α -carotene but this possibility is not consistent with the observed mixed melting-point of these two pigments (156° compared with 172° for *neo*- α -carotene). Mixed melting-point data on various binary mixtures of the three isomeric α -carotenes are given below and this lends support to the other evidence of the reality of the differences between the three isomerides.

	Melting-point (uncorrected)
α -Carotene	179°
<i>neo</i> - α -Carotene	172°
Mixture	161°
α -Carotene	180°
<i>pseudo</i> - α -Carotene	165°
Mixture	156°
<i>neo</i> - α -Carotene	172°
<i>pseudo</i> - α -Carotene	165°
Mixture	153°

The most obvious mechanism that can be suggested for the isomerization of both α - and β -carotenes on adsorption, is the displacement of the β -ionone double bond out of conjugation. In this way β -carotene would yield *pseudo*- α -carotene, having a β -ionone ring at one end of the molecule and an α -ionone ring at the other, as in α -carotene (cf. Formula I) whilst α -carotene would yield *neocarotene*, with an α -ionone residue at each end of the molecule. This hypothesis is supported by the change in the absorption spectrum and the decreased strength of adsorption on alumina after isomerization, both of which changes are perfectly consistent with a decrease of the conjugated system by one ethylene linkage. The simplest test of this hypothesis would seem to be the biological test for vitamin A potency, because *pseudo*- α -carotene

should be only half as active as β -carotene whilst *neocarotene* should be completely inactive. In actual fact the results show that *pseudo- α -carotene* is as active as β -carotene whilst *neocarotene* is definitely active (possibly having as much activity as α -carotene). These facts appear to negative the suggested mechanism but it may well be that, as the new carotenes are so labile that they can be easily reconverted into their parent compounds by simple adsorption, they are also labile enough to be reconverted into the biologically active β - and α -carotenes, respectively, in the body of the rat.

On the other hand it is possible that these isomerizations are only changes into different geometrical isomerides. There is some support for this hypothesis in the facts that like β -carotene and *pseudo- α -carotene* the *cis*- and *trans*-bixins differ slightly in the location of their absorption maxima and moreover are separable chromatographically [Kuhn & Winterstein, 1932; Winterstein, 1934]. The differences between the bixin isomerides are, however, much less than are observed with these new carotene isomerides.

Whatever be the mechanism of the isomerization under discussion it is still difficult to see why, when *neocarotene* crystallizes and is changed in the process, it gives *neo- α -carotene* and not α -carotene.

SUMMARY

Following up previous work in which it was shown that β -carotene on repeated chromatographic adsorption on alumina changes into a new pigment, *pseudo- α -carotene*, the effect of repeated adsorption of α -carotene has been studied.

It has been found that this pigment also changes into another compound, *neocarotene*, with absorption maxima at 501 and 470 m μ in CS₂ as compared with 508 and 477 m μ for the original α -carotene.

When attempts were made to obtain the *neocarotene* solid it was found that the crystals on being redissolved showed the absorption spectrum of the original α -carotene. An examination of the properties of the crystals has demonstrated that, although similar to those of α -carotene, they are in fact different, thus characterizing a new isomeride of α -carotene which has been named *neo- α -carotene*. This is similar to α -carotene in appearance, absorption spectrum and biological activity (vitamin A), but differs in melting-point and mixed melting-point, rotation and behaviour on adsorption. It is probably a geometrical isomeride of α -carotene.

The results of biological tests for vitamin A-activity on *neocarotene*, *neo- α -carotene* and *pseudo- α -carotene* are recorded.

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REFERENCES

- Gillam & El Ridi (1935). *Nature, Lond.*, **136**, 914.
— (1936). *Biochem. J.* **30**, 1735.
Jackson & Jones (1936). *J. chem. Soc.* 895.
Karrer, Morf & Walker (1933). *Helv. chim. Acta*, **16**, 975.
Kuhn & Winterstein (1932). *Ber. dtsch. chem. Ges.* **65**, 646.
Moore (1930). *Biochem. J.* **24**, 692.
Smith (1932). *J. biol. Chem.* **96**, 35.
Winterstein (1934). *Handb. Pflanzenanalyse*, **4**, 1414.