80. Palm Oil Carotenoids

2. The Isolation of Lipoid Pigments from a West African Plantation Oil and some Remarks on the Isomerization of Carotenoids

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As already mentioned [Hunter & Scott, 1941], it is to be anticipated that the carotenoids present in palm oils will vary to some extent with the district of origin and the previous history of the oils concerned. In general, African palm oils may be classified into two main groups: (i) oils of 'native' origin, and (ii) plantation oils of low free fatty acid content which are subject to greater care in production. Those of the second class might therefore be expected to show much smaller variations than the former [Strain, 1941], and to obtain information with regard to the carotenoids of an oil of the second type, a study has been made of a palm oil from a West African plantation.

A number of samples of oil from the N'dian Oil Palm Estate, which were specially imported in small amounts for this purpose, were examined as described in the first paper. Chromatographic adsorption of the petroleum-soluble unsaponifiable matter of the first of these samples of oil on a Tswett column of alumina gave a series of coloured zones, the lower two of which furnished crystalline and reasonably pure specimens of α - and β -carotene. The upper zones yielded carotenoids having the spectroscopic characteristics of lycopene and neolycopene, which exhibited the reversible isomerization described by Zechmeister & Tuzson [1938].

The ether-soluble unsaponifiable matter on being dissolved in light petroleum and partitioned with 90% methanol gave a hypophasic fraction which on chromatographic adsorption on alumina furnished a small amount of lutein. The absorption maxima of the gum obtained from the coloured zone above this corresponded with those of neolutein [Zechmeister & Tuzson, 1938].

The sterol fraction of the ether-soluble unsaponifiable matter also yielded a small amount of ergosterol, which was recognized from its ultra-violet absorption spectrum. This observation seems of particular interest in view of the presence of ergosterol in the unsaponifiable matter of grass [Gillam, Heilbron, Morton, Bishop & Drummond, 1933], soya bean oil [Izume, Yoschimaru & Hikada, 1933], cotton seed oil [Windaus & Bock, 1937], and corn germ oil [Lobert, 1938].

From the petroleum-soluble unsaponifiable matter of a second palm oil of slightly lower free fatty acid content but similar spectroscopic absorption, in addition to the isolation of a small specimen of lycopene of high extinction coefficient, evidence was obtained of the presence of γ -carotene.

With regard to the view of Zechmeister & Tuzson that the reversible isomerization of carotenoids is due to 'mobile' *cis-trans* isomerism, attention has already been drawn [Jones, 1940] to the fact that in the cases of the polyene acids, bixin and crocetin and their derivatives which are known to exist in *cis* and *trans* modifications [Kuhn & Winterstein, 1932], the more labile *cis* isomers show absorption spectra maxima displaced towards the region of shorter wave-length to an extent comparable with those observed in *neo*-carotenoids. Mulliken [1939] considers, moreover, that the conjugation of the carbonyl group and doubly linked carbon groups exerts a special stabilizing influence in such cases. Quantum mechanical considerations indicate that conjugated systems tend

to remain coplanar [Pauling, 1940], and Mulliken [1939] concludes that the ordinary and more stable form of carotenoids is the most elongated structure with *trans-trans* linkages [cf. Zechmeister, Le Rosen, Went & Pauling, 1941]. The labile *neo*-isomers presumably contain at least one *cis-trans* linkage.

The analogy with azobenzene [Hartley, 1938], although differing in that the *cis* isomer is not coplanar [Robertson, 1939] owing to steric hindrance, seems highly suggestive. The ordinary form of azobenzene whose *trans* configuration follows from its zero dipole moment [Bergmann, Engel & Sandor, 1930] and the X-ray analysis of the crystal structure [Robertson, Prasad & Woodward, 1936; de Lange, Robertson & Woodward, 1939], may be converted into a labile *cis* form which can be separated chromatographically [Cook, 1938]. Dilute solutions of the same concentration of either isomer furnish an equilibrium mixture containing 15–40% of the *cis* form, depending on the solvent. It is noteworthy that all the carotenoid isomerizations so far recorded have taken place in non-polar solvents, in which Hartley [1938] observed rapid isomerization of azobenzene. We have now shown that β -carotene isomerizes more rapidly in toluene (non-polar) than in nitromethane (polar) solution, thus paralleling the latter observations.

The original theory that the isomerization of carotenoids is due to three-carbon prototropy with movement of a terminal double bond out of the conjugated system [Gillam & El Ridi, 1936] is ruled out, not only by the arguments adduced by Zechmeister *et al.* [1941], but by the fact that α -carotene gives rise to an isomer, *neo*- α -carotene, with retention of optical activity [Gillam, El Ridi & Kon, 1937]. The tautomeric system is of the true three-carbon type (as distinct from the pentad keto-enol system) such as is present in the diphenylpropenes which were first thought to be static [Ingold & Piggott, 1922], and only undergo interconversion under most stringent conditions [Ingold & Shoppee, 1929].

EXPERIMENTAL

The first oil examined was a small delivery which had a free fatty acid content of 3.5% and which showed an extinction coefficient of 2.65 at 458 $m\mu$ in chloroform. A number of experiments were made with this oil, of which the following are typical.

(i) The palm oil (200 g.) was heated under reflux with a mixture of aqueous KOH (56%, 200 g.) and industrial alcohol (600 ml.) for 1½ hr. and the resulting soap solution was diluted with a further 600 ml. of alcohol, made up to 4 l. with water, and thrice extracted with light petroleum. The united extracts were thrice washed with 90% methanol (total volume 500 ml.), thereafter with water until free from alkali, dried with anhydrous Na_2SO_4 , and freed from solvent under reduced pressure in an atmosphere of nitrogen.

The petroleum-soluble unsaponifiable matter was resaponified by heating with a mixture of alcoholic NaOH (3 g. of NaOH in 250 ml. of 90% alcohol) and benzene (50 ml.) for 2 hr., and the benzene was removed by distillation. The mixture was diluted with 50% alcohol (500 ml.) and thrice extracted with light petroleum, the united extracts washed and evaporated, and a solution of the residue in light petroleum was adsorbed on a Tswett column of 'Birlec' alumina. The chromatogram furnished a narrow dark red zone (1), below the usual 'oxidized carotenoid zones', whose appearance was suggestive of lycopene, followed by a second narrow orange brown zone (2) which separated from the broad carotene zone (3). Elution of zone (1) gave 0.004 g. of a carotenoid which showed absorption maxima at 539, 501 and 469 $m\mu$ in carbon disulphide and evidently consisted of impure lycopene. Zone (2) furnished 0.009 g. of material showing absorption maxima at 526, 491.5 and 462 $m\mu$ in carbon disulphide and a band at 590 $m\mu$ in the antimony trichloride reaction. The carotene zone (3) on being rechromatographed on Merck's alumina furnished crude β -carotene whose solution in ethyl acetate furnished crystals, M.P. 158°, on keeping in a refrigerator. On recrystallization these gave glistening red-brown crystals, M.P. 181–182°, which showed absorption maxima at 516 and 485 $m\mu$

in carbon disulphide and at 495 and 463 $m\mu$ in chloroform. The $E_{1\text{cm}}^{1\%}$ value at 463 $m\mu$ in chloroform was 2080.

(ii) The unsaponifiable matter from 3 kg. of the oil was worked up in batches as follows. The oil (1000 g.) was heated with NaOH (33 %, 840 g.) and industrial alcohol (3.3 l.), under reflux, for an hour and the solution was diluted with alcohol (3 l.) and water (12.6 l.). 4 l. of a mixture of light petroleum and methylated ether (1 : 1) were added and the mixture was introduced into an extraction column packed with $1\frac{1}{2}$ in. Lessing porcelain rings and the extraction was continued for 4 hr. The united extracts were washed and the residue obtained by removal of solvent was resaponified by heating with a mixture of alcoholic NaOH (10 g. of NaOH in 400 ml. of 90 % alcohol) and benzene (80 ml.) for 5 hr. The residue obtained by removal of benzene was extracted five times with light petroleum in the presence of 50 % aqueous alcohol (1000 ml.), and the united extracts were washed with 90 % alcohol and water and dried with anhydrous Na_2SO_4 . The unsaponifiable matter obtained by removal of solvent weighed 7.49 g.

On treatment with a small amount of acetone, the unsaponifiable matter deposited a solid 'A' (0.92 g.). The filtrate from this on evaporation and adsorption of a solution of the residue in light petroleum on a Tswett column of 'Birlec' alumina gave a series of narrow upper zones (orange-brown, red-brown, orange-brown, orange-red, orange-yellow, orange-red, and yellow, in order of decreasing adsorption), all of which gave substances showing indefinite absorption spectra in the visible region, followed by a broad orange-red zone containing lycopene. Extraction of this with acetone furnished a solid 'B' (0.2 g.) which showed maxima at 546, 507, 474 and 452 $m\mu$ in carbon disulphide and at 520, 485, 455 and 427 $m\mu$ in chloroform, in agreement with recorded data for lycopene.

The orange-yellow *neolycopene* zone below this furnished 0.167 g. of a substance whose solution in benzene-methanol gave, however, a small amount of material showing the spectroscopic characteristics of lycopene (maxima at 545, 505 and 475 $m\mu$ in carbon disulphide and at 516, 483 and 457 $m\mu$ in chloroform). The mother liquors from this, on evaporation and further chromatography on 'Birlec' alumina also gave material having the spectroscopic characteristics of lycopene (maxima at 543, 505 and 475 $m\mu$ in carbon disulphide). The mother liquors from the original lycopene extract on evaporation and chromatography on 'Birlec' alumina furnished two main zones, the upper one of which had an orange-pink colour and furnished lycopene (maxima at 545, 507 and 476 $m\mu$ in carbon disulphide). The lower orange-coloured zone gave a substance showing maxima at 536, 499 and 472 $m\mu$ in carbon disulphide. Zechmeister & Tuzson [1938] record 536, 498 and 466 $m\mu$ for the absorption maxima of *neolycopene* in carbon disulphide.

The broad carotene zone below *neolycopene* consisted of a mixture of α - and β -isomers in which the latter predominated. The solid 'A' which originally separated on treatment of the unsaponifiable matter with acetone consisted mainly of a mixture of α - and β -carotenes. A solution of this in light petroleum on adsorption on a column of Merck's alumina gave a chromatogram consisting of a small narrow dark red upper zone, followed by a broad typical β -carotene zone and a smaller lighter coloured zone containing the α -isomer. On elution with acetone the β -carotene zone furnished crops of crystals melting at 182° and 179°.

(iii) The oil (1500 g.) was saponified in two batches of 750 g. as described for 'Sherbro' palm oil in our first paper. The heat of the reaction enabled a temperature of about 44° to be maintained without external heating for some time. The united petroleum extracts (ca. 1500 ml.) were washed, dried and evaporated under reduced pressure in an atmosphere of CO_2 , the temperature being kept below 50°.

The extracted soap solution was diluted with water (3350 ml.) and extracted with ether. The ether-soluble unsaponifiable matter (3.53 g.) was dissolved in light petroleum (250 ml.) and the solution extracted 10 times with 90 % methanol (total volume 500 ml.); the united extracts were diluted with water (1000 ml.) and thrice extracted with ether.

The residue obtained by removal of ether was dissolved in light petroleum and adsorbed on a column of 'Birlec' alumina, and the chromatogram was developed with petroleum containing 0.5 % of methanol. Four zones were obtained (orange, yellow, orange-brown, and red-brown in order of decreasing adsorption) which were separated, eluted and separately re-chromatographed. The carotenoid from the upper zone of the original chromatogram now gave a well-defined main zone on being developed with light petroleum containing 3 % of benzene. On elution this gave a small amount of solid which showed maxima at 507.5, 476 and 447 $m\mu$ in carbon disulphide and at 489, 458.5 and 431 $m\mu$ in chloroform. The antimony trichloride reaction gave a maximum at 620 $m\mu$ which shifted to 623 $m\mu$, and a solution of the carotenoid also gave a blue colour in conc. H_2SO_4 which turned green on pouring into water [Zechmeister, 1934]. Gilman [1938] gives the absorption maxima for lutein in carbon disulphide as 508, 475 and 445 $m\mu$, and Gillam [1935] recorded the maxima of lutein (from nettles) in chloroform as 489, 456 and 428 $m\mu$.

The second zone furnished a gum which showed absorption maxima at 502, 473 and 442 $m\mu$ in carbon disulphide and at 484, 454 and 431 $m\mu$ in chloroform. A solution of the gum in methyl alcohol furnished a trace of solid which gave absorption maxima at 508, 477 and 446 $m\mu$ in carbon disulphide and at 488, 457 and 430 $m\mu$ in chloroform. Zechmeister & Tuzson [1938] record maxima at 501 and 468 $m\mu$ for *neolutein* A and at 502 and 470 $m\mu$ for *neolutein* B in carbon disulphide. The third zone of the original chromatogram gave no substance of distinctive properties. The fourth zone (red-brown) on being re-chromatographed furnished a gummy solid which showed a vague maximum at 475 $m\mu$ in carbon disulphide, and vague maxima at 487 and 460 $m\mu$ in chloroform.

The petroleum-soluble unsaponifiable matter (4.33 g.) was resaponified at 40° for 4 hr. and its solution in light petroleum was thereafter adsorbed on a column of 'Birlec' alumina. The dark red zone above the main carotene zone on being re-chromatographed furnished a small amount of crystalline lycopene (absorption maxima at 547, 507 and 478 $m\mu$ in carbon disulphide and at 520, 485 and 455 $m\mu$ in chloroform). The main carotene zone on further chromatography on alumina ('Birlec' and thereafter British Drug Houses Ltd., using a 20 % solution of benzene in light petroleum for development) furnished α -carotene which separated from benzene-methanol in crystals melting at 184°. This had an $E_{1\text{cm}}^{1\%}$ value of 2270 at 460 $m\mu$ in chloroform and a rotation of $[\alpha]_D = +600^\circ$ in benzene.

The first part of the percolate obtained during chromatography of the main carotene zone furnished a small amount of waxy material which separated from acetone in colourless crystals, m.p. 54° which rose to 57–58° on recrystallization, and which was evidently hentriacontane [Hunter & Scott, 1941].

(iv) In an experiment similar to (i) in which 500 g. of the palm oil were saponified by heating with aqueous KOH (56 %, 500 g.) and industrial alcohol (1500 ml.) for an hour, the ether-soluble unsaponifiable matter was dissolved in light petroleum to give a solution which deposited an orange-coloured powdery material (m.p. ca. 150°) on keeping. The filtrate from this, on adsorption on alumina, gave a series of zones (yellow, brown, orange, red-brown, pale orange, etc. in order of decreasing adsorption), the second of which, on extraction with acetone, furnished crops of pale yellow crystals, m.p. 139° and m.p. 140°. The mother liquors on evaporation and crystallization of the residue from light petroleum gave a further crop of crystals, m.p. 137°. The combined crops on recrystallization from acetone furnished transparent plates, m.p. 139.5°. A solution of this substance in alcohol showed the ultra-violet absorption spectrum of ergosterol with maxima at 250.5, 260.5, 270, 281 and 293 $m\mu$. Hogness, Sidwell & Zscheile [1937] record absorption maxima at 252, 262, 271, 282 and 293 $m\mu$ for ergosterol in ethyl alcohol. The $E_{1\text{cm}}^{1\%}$ value at 281.5 $m\mu$ was, however, only 7.7, indicating the presence of only some 2.5 % of ergosterol.

The residue obtained by evaporation of the mother liquors from the acetone solution, on being dissolved in alcohol and treated with digitonin in the same solvent, gave a precipitate which was washed with alcohol and ether and thereafter heated, under reflux, with acetic anhydride (25 ml.) for an hour. The residue obtained by removal of acetic anhydride by distillation, on recrystallization from dilute alcohol ('norit') furnished crystals melting at 131°.

The second palm oil examined was a 5 cwt. drum delivery which had a free fatty acid content of 2.8 % and showed an extinction coefficient of 2.6 at 458 m μ in chloroform.

1500 g. of this oil were saponified in two batches as in (iii) and the petroleum-soluble unsaponifiable matter was adsorbed on 'Birlec' alumina, using a 25 % solution of benzene in light petroleum as solvent and for development. The chromatogram formed a series of zones: (1) brown, (2) dark red, (3) red-brown, (4) ochre, (5) typical carotene and (6) narrow pale yellow, in order of decreasing adsorption. The lowest zone and part of the carotene zone were washed out of the Tswett column. Evaporation of the former and crystallization of the residue from acetone gave a small amount of hentriacontane melting at 67–68°. The brown zone (1) yielded material showing indefinite absorption in carbon disulphide. Zone (2) on being re-chromatographed on 'Mayfair' alumina, using 50 % benzene in light petroleum for development of the new chromatogram gave a main zone containing lycopene. A small specimen of this which crystallized from benzene-methanol, although it had a low melting point (160°), showed well-defined absorption maxima at 518, 485 and 454 m μ in chloroform with the corresponding $E_{1\text{cm.}}^{1\%}$ values of 2070, 2380 and 1610. In cyclohexane, it showed maxima at 509, 476 and 448 m μ with $E_{1\text{cm.}}^{1\%}$ values of 2100, 2420 and 1620 respectively. Gillam [1935] recorded a figure of only 2000 for the $E_{1\text{cm.}}^{1\%}$ value of lycopene at its main absorption band in chloroform. The smaller zone below this furnished a carotenoid showing maxima at 535, 496 and 465 m μ in carbon disulphide, which was probably neolycopene.

Zone (3) on being re-chromatographed on 'Mayfair' alumina gave a main orange-red zone which furnished a carotenoid showing absorption maxima at 529, 494 and 463 m μ in carbon disulphide which was probably γ -carotene [Hunter & Scott, 1941]. A dark red zone which formed below this gave a substance having almost identical spectroscopic characteristics (maxima at 529, 494 and 458 m μ).

Zone (4) on being re-chromatographed gave a yellow zone between more coloured zones which furnished a carotenoid showing maxima at 454 and 426 m μ in carbon disulphide.

The ether-soluble unsaponifiable matter was dissolved in light petroleum and the solution was extracted with 90 % methanol as in previous experiments. On adsorption on 'Birlec' alumina, using 50 % benzene in light petroleum, a chromatogram consisting of five zones was obtained (brown-orange, narrow brown, red, orange and yellow, in order of decreasing adsorption). Elution of the first three of these gave substances having very indefinite absorption spectra, but the orange zone below them gave a carotenoid showing absorption maxima at 503 and 472 m μ in carbon disulphide. This gave a purple-blue colour without selective absorption in the antimony trichloride reaction. The yellow zone below this yielded a carotenoid showing maxima at 502 and 473 m μ in carbon disulphide, which gave a transient band at 590 m μ in the antimony trichloride reaction.

Experiments on the isomerization of β -carotene

Crystalline β -carotene (ca. 5 mg.) was dissolved in carbon disulphide (ca. 0.8 ml.); 0.15 ml. of this solution was added to nitromethane (2 ml.) and the mixture was heated in a water bath with constant shaking for 3 min. The solution was then rapidly evaporated to dryness on a Hyvac pump fitted with a CO₂ trap, and the residue was dissolved in carbon disulphide and examined on the Hilger-Nutting spectrophotometer.

A similar experiment was made with 0.15 ml. of the β -carotene solution in carbon disulphide and 2 ml. of toluene.

The results were as follows:

	Absorption maxima m μ	
Original β -carotene solution	516,	485
After treatment with nitromethane	505-510,	483
After treatment with toluene	—	477-478

The solutions of carotene which had been treated with nitromethane and toluene were evaporated and the residues chromatographed on 'Mayfair' alumina, using a 20 % solution of benzene in light petroleum as solvent. The carotene which had been treated with nitromethane yielded three zones (narrow orange, pink, and orange) which on elution gave substances with the following spectroscopic characteristics in carbon disulphide:

	Absorption maxima m μ
Zone 1	494
Zone 2	510, 483
Zone 3	510, 483

The carotene which had been treated with toluene gave two zones of similar appearance to zones 1 and 3, but only the second of these gave sufficient material for examination, and showed maxima at 505-510 and 481 m μ in carbon disulphide.

SUMMARY

1. Examination of the unsaponifiable matter of a West African plantation oil of low free fatty acid content showed the presence of α -, β - and γ -carotenes, lycopene, *neolycopene*, lutein, *neolutein*, and the carotenoid intermediate between γ - and β -carotenes on the chromatogram which was previously observed in 'Sherbro' palm oil.
2. Evidence was also obtained of the presence of ergosterol.
3. Analogy is drawn between the reversible isomerization of carotenoids and the 'mobile' *cis-trans* isomerism of azobenzene.

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