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Prolycopene

BY A. L. LEROSEN AND L. ZECHMEISTER

Until a few years ago no data were available concerning stereoisomerism in the class of the natural C_{40}-carotenoids which are supposed to possess an all-*trans* configuration. Gillam and El Ridi[1] were the first to notice in the course of repeated chromatographic experiments that in the Tswett column β-carotene may develop a second, paler pigment layer which shows a spectrum of markedly lower wave lengths than the starting material. A migration of a double bond or possibly a *trans–cis* change was taken into consideration. While the authors mentioned attributed the phenomenon to the influence of the column itself, it was later shown[2] that fresh solutions of carotenoids on standing generally undergo a spontaneous isomerization the rate of which increases under the influence of higher temperature or iodine catalysis. A partial *trans–cis* shift has been indicated as the explanation of this reversible conversion, which has nothing to do with the chromatographic experiment itself.

The wave lengths of the absorption maxima of the "neo"-carotenoids formed were 5–20 mμ shorter than those of the respective parent pigment in petroleum ether solution. Most carotenoids tested have given rise to from one to three such isomers.

It was at first assumed that these isomers do not occur in nature. Recently, however, it has been reported by Zechmeister, LeRosen, Went and Pauling[3] that the pigment mixture occurring in the ripe tangerine tomato (a variety of *Lycopersicum esculentum*) contains as main polyene a new carotenoid, prolycopene, the spectrum of which, in petroleum ether, is 35 mμ lower than that of lycopene, $C_{40}H_{56}$. Similar differences are observed in other solvents as summarized in Table I. On addition of iodine a striking change occurs in the spectrum, *viz.*, the bands typical for lycopene appear immediately. The new spectrum is, in fact, that of a complex equilibrium mixture of stereoisomers in which lycopene prevails.

According to Pauling[4] only seven conjugated double bonds of the eleven contained in lycopene are available for *cis–trans* isomerization, allowing 72 stereoisomers.[3] It is assumed that at least five *cis* double bonds are contained in prolycopene.

While in the first investigation prolycopene was described only in solutions, obtained by chromatography of the extract of a single fruit, recently more than 600 mg. of the pure crystalline pigment was isolated. Prolycopene shows many characteristics of the well-known carotenoids. Its color intensity is about half that of lycopene in dilute petroleum ether solution (Pulfrich photometer, light filter S 47). It can be recrystallized from petroleum ether and methanol. The crystals (Fig. 1) are markedly different from those of lycopene. Under the microscope dull brown crossing sections of crystal individuals are noticed in contrast to the corresponding intensely red color of lycopene. The crystals bleach in air within a few days, *i. e.*, much more rapidly than lycopene. In one instance about 18 atoms of oxygen were added.

Prolycopene is not adsorbed by calcium carbonate. On the calcium hydroxide column it is fixed easily and is located below lycopene, γ-carotene or cryptoxanthin but above β-carotene and the stereoisomers of the latter.

In solution the stability of pure prolycopene, $C_{40}H_{56}$, is by no means inferior to lycopene provided that certain catalysts are carefully excluded. Even if refluxed in petroleum ether for 30 min. very slight isomerization occurs which can be demonstrated in the Tswett column. If it is, however, kept above its melting point (111°) for a few minutes in a sealed tube filled with carbon dioxide and rapidly cooled to room temperature,

(1) A. E. Gillam and M. S. El Ridi, *Nature*, **136**, 914 (1935), *Biochem. J.*, **30**, 1735 (1936); A. E. Gillam, M. S. El Ridi and S. K. Kon, *ibid.*, **31**, 1605 (1937); G. Ph. Carter and A. E. Gillam, *ibid.*, **33**, 1325 (1939). Two stereoisomeric forms of bixine $C_{25}H_{30}O_4$ and of crocetin $C_{20}H_{24}O_4$ had been described earlier by J. Herzig and F. Faltis, *Ann.*, **431**, 40 (1923); P. Karrer, A. Helfenstein, R. Widmer and Th. B. van Itallie, *Helv. Chim. Acta*, **12**, 741 (1929), and by R. Kuhn and A. Winterstein, *Ber.*, **66**, 209 (1933) and **67**, 344 (1934).

(2) L. Zechmeister and P. Tuzson, *Nature*, **141**, 249 (1938), *Biochem. J.*, **32**, 1305 (1938), *Ber.*, **72**, 1340 (1939); L. Zechmeister, L. von Cholnoky and A. Polgár, *ibid.*, **72**, 1678 and 2039 (1939); *cf.* H. H. Strain, "Leaf Xanthophylls," Carnegie Inst. of Washington, Publ. No. 490 (1938).

(3) L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Nat. Acad. Sciences*, **27**, 468 (1941); D. Nagy, *Iowa State Coll. J. Sci.*, **15**, 89 (1940), (and Thesis) claimed that neozeaxanthins occur naturally; no experimental data are given.

(4) L. Pauling, *Fortschr. Chemie organ. Naturstoffe*, **3**, 203 (1939).

TABLE I

ABSORPTION MAXIMA OF FRESHLY PREPARED SOLUTIONS OF PROLYCOPENE AND LYCOPENE CRYSTALS (mμ)

Parentheses denote blurred bands

	Prolycopene			Lycopene			
Carbon disulfide	500.5	469.5	(440)	547.5	508.5	476	(449)
Pyridine	487.5	456	(429)	529	492.5	460.5	
Benzene	485	455.5	(429)	523	488.5	458	(430.5)
Chloroform	484	453.5		521.5	487.5	457	(423)
Carbon tetrachloride	483.5	453	(427)	521	486.5	456.5	(431.5)
Dioxane	479	449		515.5	484	453.5	(430)
Acetone	(472)	(445)		507.5	476.5	448.5	
Ethyl alcohol	(471)	(445)		505.5	475	447.5	
Petroleum ether	470	443.5		505	474	446	
Ether	(468.5)	(443)		505	474	445.5	
Methyl alcohol	(467)	(443)		502	472	445	

the chromatogram shows about a dozen layers. Prolycopene predominates and is immediately followed by an interesting new pigment showing maxima at still shorter wave lengths, *viz.*, at 464 and 438 mμ, in petroleum ether. All the other pigments which also include lycopene are adsorbed above prolycopene.[5]

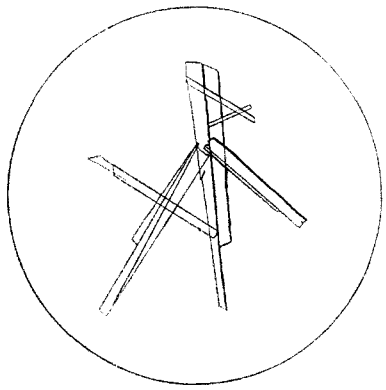

Fig. 1.—Prolycopene crystallized from petroleum ether and ethyl alcohol (border lines of crystals were irregular as drawn).

The isomerization of prolycopene under the catalytic influence of iodine is described below. The numerous colored layers obtained in this way belong to three distinct groups with markedly different adsorption affinities (Table III). They all must be classified as stereoisomers of lycopene and prolycopene: if each layer is cut out separately, eluted, transferred into petroleum ether and treated with iodine, similar equilibrium mixtures are obtained, possessing maxima at 502.5, 471.5 and 443 mμ. Adsorption analysis shows the presence of substantial amounts of lycopene accompanied by a series of minor pigments.

Sulfur and hydrobromic acid have already been mentioned as catalysts.[3] Hydrochloric acid in petroleum ether is effective but it acts much more slowly than iodine. Light is also effective.

Carotenoids of the described "pro"- or "*cis*"-type seem to be widespread in nature, especially in some fruits, as shown by unpublished experiments. While the qualitative pigment analysis as used in this Laboratory has so far included only a simple spectroscopic examination of the chromatographic layers, each individual pigment is now tested before and after the addition of iodine in the spectroscopic cell. All-*trans* carotenoids show a slow decrease of the wave lengths. If, however, a rapid and substantial increase is noticed or if a new band appears in the region near the longer wave lengths, the pigment should be classified as belonging to the pro-series.

In tangerine tomato extracts the main pigment, prolycopene, is accompanied by a number of minor carotenoids (Table II), some of which have been crystallized and are under investigation.

Acknowledgment.—We wish to thank Professor F. W. Went, who drew our attention to the fruit examined, Dr. J. Lesley of the Citrus Experiment Station in Riverside, California, for providing us with starting material and Dr. G. Oppenheimer and Mr. G. Swinehart for microanalytical assistance.

Experimental

Materials and Apparatus.—The adsorbent used throughout the following experiments was calcium hydroxide (Shell Brand lime, chemical hydrate; 98% through 325 mesh). The average particle diameter was about 7–14 μ. The rate of flow through a column 150 × 17 mm. was 11 mm./min. under a 30-mm. vacuum. The chromatographic tubes were as described by Zechmeister and Cholnoky[6] and manufactured by Scientific Glass Appara-

(5) The behavior of some other carotenoids in melt is under investigation.

(6) L. Zechmeister and L. Cholnoky, "Die chromatographische Adsorptionsmethode," 2nd ed., Julius Springer, Vienna, 1938; "Principles and Practice of Chromatography," facing p. 62, Fig. 19, John Wiley and Sons, Inc., New York, N. Y., 1941.

TABLE II

CAROTENOIDS IN THE EXTRACT OF TANGERINE TOMATO PULP, LISTED IN THE SEQUENCE OF DECREASING ADSORBABILITY ON THE CALCIUM HYDROXIDE COLUMN

Zone	Mg pigment in 100 g. of dried pulp[a] (7300 g. fresh pulp)	Color of adsorbate	Spectrum in petroleum ether (mμ) Before The addition of iodine			After		
1	1.24	Brownish	No definite bands					
2	3.28	Faint pink	497	465.5	(432.5)	496.5	462	431
3	14.41	Red	503	473.5	447.5	502	470	442
4	12.8	Red	500.5	469	440.5	502	470	442
5	7.0	Orange-red	497.5	468.5	(440.5)	500	470	442
6	42.0	Orange		476.5	447	502	470	442
7	41.6	Yellow		473	444 (417)	502	470	442
8	55.0	Orange		471	443	502	470	442
9, 10	21.2	Orange / Yellow		468	439.5	500	469.5	442.5
11	9.85	Light pink	494.5	464.5	438	498	468	440
12	24.0	Yellow		471.5	441.5	500.5	470	442
13, 14	162.0[b]	Pink-orange / Orange		471	442	502	470	442
15	9.5	Orange-yellow		465	436.5	502	470	442
16	53.2	Yellow		466	438	502.5	471.5	441.5
17	1.31	Yellow			431			430
18, 19, 20	37.7	Yellow / Orange / Yellow			430			430
21	7.75	Yellow			427.5			430.5
22	1.46	Faint orange	(486)	(453.5)	426.5			430
23	5.55	Faint orange	486.5	455.5		486.5	455.5	

[a] For the estimation of the dry weight a separate small sample was dehydrated with methanol, filtered on a Buchner funnel and dried at 40° for twenty-four hours. [b] Prolycopene (no. 14); band 13 was either due to prolycopene spreading on the column, or another polyene present in very small amount.

tus Co. in Bloomfield, N. J. The petroleum ether (b. p. 60–70°) was "Skelly-solve." For elutions, sintered and glass funnels (Jena 11G3 to 26G3) were used. The eluates were washed free from alcohol in a continuous washing apparatus as described by one of the authors.[7] The spectra were determined in an Evaluating Grating Spectroscope as devised by Loewe and Schumm (Zeiss, light filter Jena BG7) and the concentration of pigments in the Pulfrich Gradation photometer (Zeiss, light filters S 43, S 47, and S 50 used as indicated below); photometric values as given by Cholnoky (in print) except for prolycopene.

Estimation of the Pigment Components.—One hundred grams of fresh pulp (without skin and seeds) was ground in a mortar under methyl alcohol, transferred to a glass stoppered bottle and after the addition of 0.25 vol. of petroleum ether mechanically shaken for fifteen minutes. After the liquid had been filtered off the residue was ground again and shaken with a 1:3 methanol–petroleum ether mixture. These operations were repeated twice. By addition of water the pigment was quantitatively transferred into petroleum ether which was dried with sodium sulfate, concentrated under diminished pressure and chromatographed on a calcium hydroxide column (24.5 × 4 cm.). In some cases several layers were too close to separate and therefore were determined together. Each layer was eluted with ethyl alcohol + petroleum ether (1:3) and transferred into petroleum ether for spectroscopic and photometric examination. The photometric values for the polyenes possessing a first band near 500 mμ were calculated as "lycopene," and for those having this maximum around 470 mμ as prolycopene (light filter S 47). If the first band was located near 430 mμ the light filter S 43 was used and the values were interpreted as "lycopene" on the basis of the data valid for the filter S 47. The latter approximative procedure was satisfactory only because of the small absolute amounts. The results are summarized in Table II.

Isolation and Properties.—For preparative experiments 30 kg. of ripe fruits were picked in September. Each tomato was cut into a few parts and ground in a meat grinder. The orange colored pulp was pressed in cheese cloth in a fruit press. Some material suspended in the juice rose to the surface on dilution with one-fourth vol. of methanol. The clear liquid was siphoned off and after the addition of more methyl alcohol the pulp was separated in the press and added to the main portion. The material was then divided into four parts each of which was treated as follows.

The pulp was transferred to a 4-liter bottle and almost covered with methyl alcohol. After the addition of one-third volume of petroleum ether it was shaken for twenty minutes and transferred to the press. The liquid obtained consisted of two layers. The pulp was extracted six more times in a similar way but the ratio methanol-petroleum ether was 1:1 in the second extraction and 1:10 in the others. The extracts (from the 30 kg. of fruit) were combined in a 15-liter separatory funnel. Water was added in

(7) A. L. LeRosen, *Ind. Eng. Chem., Anal. Ed.*, **14**, 165 (1942).

a volume about equal to the slightly colored methyl alcoholic layer. The lower phase was then discarded and the upper washed methanol free.

The pigment solution was dried with sodium sulfate and concentrated *in vacuo* to about 1 liter in a slow stream of carbon dioxide. It was divided into two halves, each of which was diluted with 1 vol. of petroleum ether and chromatographed on calcium hydroxide in a conic percolator (48 × 20 × 8 cm.). The chromatogram consisted of the following main sections, after having been developed with 4 liters of petroleum ether containing 10% acetone: four pink zones (near the top), two orange ones and a very broad orange prolycopene layer which was closely followed by a bright yellow zone and finally by β-carotene (and some of its stereoisomers).

The prolycopene was cut out, eluted with an alcohol–petroleum ether mixture (1:3) from which the pigment was transferred into the latter solvent by addition of water. The thoroughly washed and dried solution (1.5 to 2 liters) when evaporated *in vacuo* left a partially crystalline residue which was dissolved in the smallest possible volume of petroleum ether. Gradually 95% ethyl alcohol was added at room temperature until the first crystals appeared. The liquid was kept at 25° for one-half hour and then at 5° overnight. The glittering red crystals were filtered off on sintered glass and washed with methanol. The yield was 618 mg. = 93% of the prolycopene content in 30 kg., or 20.6 mg. per kg. of fresh fruit. A 5-kg. experiment yielded 18.7 mg. per kg.

As the sample was chromatographically homogeneous (with exception of a minute yellow streak below due to isomerization) it was dried in high vacuum and directly analyzed.

Anal. Calcd. for $C_{40}H_{56}$: C, 89.48; H, 10.52; mol. wt., 536. Found: C, 89.67; H, 10.61; mol. wt., 528 (in exaltone; micro method of Rast).

For substances of this type the melt from the molecular weight determination should be chromatographed in order to estimate the extent of isomerization. In one experiment the prolycopene–exaltone mixture has been melted only twice and this sample showed in the Tswett column negligible amounts of isomerized pigment. In another case, however, in which the melting was repeated seven times, more than half of the starting pigment was changed, mostly into lycopene.

Prolycopene is much more soluble than lycopene, especially in cold petroleum ether. The solubility of both pigments in methanol is very poor at room temperature. Prolycopene can best be recrystallized from petroleum ether on addition of methanol. The microscope shows long flat prisms up to 0.5 mm. in length. Most crystals do not show the swallow-tail-like ends characteristic for lycopene. They are generally grouped at random and occasionally in star-like aggregates. In some instances broad plates appeared. The melting point is 111° (cor., Berl block, raise of temperature 3° per min., the sample was introduced 30° below the melting point).

The color of the calcium hydroxide adsorbate (from petroleum ether) is dull orange while lycopene is red and β-carotene less brownish than prolycopene. The three hydrocarbons can be separated easily by washing the column with petroleum ether containing 5 to 10% acetone, depending on the quality of the lime.

The extinction coefficient (k) of prolycopene in petroleum ether has been determined in the Pulfrich photometer (light filter S 47; c = mg. pigment in 100 cc. of solution):

k	0.2	0.4	0.6	0.8	1.0
c	.14	.30	.47	.66	0.85

Isomerization Phenomena.—The influence of the ratio gram-atom iodine/mole prolycopene (in petroleum ether) may be demonstrated easily. If this ratio is 1/20,000, 1/2000 or 1/200, respectively, 2, 37 and 93% of the pigment undergoes isomerization in one minute, at room temperature. With the ratio 1/200 no unchanged prolycopene was present after thirty minutes. In some experiments a fraction of the pigment was evidently converted into colorless or faintly colored substances which will be investigated later. This became manifest by considerable losses in color intensity, up to 30% of the total pigment, as indicated by the photometric balance. In contrast, 97% of prolycopene could be recovered in blank experiments in which no catalyst was added.

The description of two typical experiments follows. (a) The solution of about 5 mg. of crystalline prolycopene in 50 cc. of petroleum ether, treated with 30 μg of iodine (in the same solvent), was after two minutes of standing rapidly sucked into a calcium hydroxide column (24.5 × 4 cm.) and developed with petroleum ether + acetone (9:1). Fourteen (or more) colored layers appeared; they were roughly grouped in three sections (Table III) of which only the first showed a maximum round 500 mμ. In this group lycopene + neolycopene, in the third prolycopene was preponderant; the latter corresponded to the half of the total adsorbed pigments. When eluted with alcohol, transferred into petroleum ether and treated with iodine, each zone (with the possible exception of the lowest one) gave an equilibrium mixture showing maxima be-

TABLE III

THE PIGMENTS FORMED FROM PROLYCOPENE BY IODINE CATALYSIS, LISTED IN THE SEQUENCE OF DECREASING ADSORPTION AFFINITIES

The spectra are given in petroleum ether; the maxima of main layers are printed in italics; faint bands are in parentheses. The homogeneity of some of the minor polyenes is questionable.

First group:	*504.5*	*473.5*	*445.5* mμ	(lycopene)[a]
	500.5	*470*	*441*	(neolycopene A)
	498	467	438.5	
	496	464	436	
	(501)	(466)		
	497	466	(437)	
Second group:		*477.5*	*447.5*	
		473	*444*	
		469.5	441.5	
		470.5	441	
		470.5	441	
Third group:		*470.5*	*445*	(prolycopene)
		465	438	
		466	439	

[a] In some cases on long development with benzene one or two minor zones appeared just below this layer, showing the lycopene spectrum. Such (yet uninvestigated) pigments may be contained in the crude lycopene crystals obtained from prolycopene; the melting point may be considerably depressed.

tween 500, 470, 441 mμ and 502, 471.5, 442 mμ. (b) To 2.6 mg. of prolycopene in 100 cc. of petroleum ether 12.5 μg. of iodine was added and the solution was chromatographed after thirty minutes of standing at room temperature as described above. The column was developed with benzene. Prolycopene had disappeared and the chromatogram showed only the following three layers which can also be obtained from lycopene by heating or on addition of iodine: (1) lycopene (top; 522.5, 488, 457 mμ), (2) neolycopene A (previously termed "neolycopene," 515.5, 482, 451.5 mμ) and (3) a new neolycopene, now termed "B" (bottom; 508.0, 477.5, 448 mμ); neolycopene A predominated.

Summary

A representative of a new class of natural C_{40}-carotenoids, prolycopene, $C_{40}H_{56}$, has been isolated in crystalline form from the ripe fruits of the tangerine tomato, a *Lycopersicum esculentum* variety. Prolycopene is the chief pigment, its quantity being about 20 mg. per kg. of fresh fruit. It can be separated chromatographically from many minor polyenes. The chromophore of prolycopene probably contains 5 to 7 *cis*-double bonds. The spectrum in different solvents is from 35 to 47 mμ shorter wave length than that of lycopene, $C_{40}H_{56}$. On addition of iodine, however, a new spectrum appears instantaneously, showing maxima which now differ from those of pure lycopene by a few millimicrons only. A mixture of numerous intermediate stereoisomers is then present which can be differentiated in the Tswett column.

PASADENA, CALIF. RECEIVED DECEMBER 8, 1941

[CONTRIBUTION OF THE COCONUT RESEARCH SCHEME, BANDIRIPPUWA ESTATE]

The Seed Fat of Litsea longifolia Bth. & Hk.

BY REGINALD CHILD AND WILFRED R. N. NATHANAEL

The seed fats of many tropical species of *Lauraceae* are characterized by an unusually high content of combined lauric acid. Of the *Litsea* genus and the closely related *Neolitsea* genus, fairly detailed accounts of the seed fats of three species are available. Puntambekar[1] has described those of *Litsea chinensis* (= *L. glutinosa*, C. B. Rob.) and *L. citrata*, both samples of Indian origin. Gunde and Hilditch[2] have recorded the fatty acid and glyceride composition of both the seed and fruit coat fats of *Neolitsea involucrata* (Nees.) Merrill (= *Litsea zeylanica*) from Ceylon.

Litsea longifolia, known in Sinhalese as Ratkeliya, is a small tree common in the moist regions of Ceylon up to 3000 ft. Trimen[3] refers to the species as *L. cauliflora*, but Alston[4] with stricter adherence to the rules of nomenclature adopted the present name, giving the following synonymy: *Litsea longifolia* Bth. & Hk. f. Gen. Pl. III, p. 161 (1883). *Tetranthera cauliflora* Moon Cat. p. 69 (1824) nomen. *T. longifolia* Nees. Syst. Laurin. p. 528 (1836).

The sample of seeds used for the present investigation was collected for us by the Forest Department in the Matara district in the South of Ceylon. One hundred seeds weighed approximately 6.7 g., and consisted of about 66% kernels and 34% seed coats. The kernels contained 9.8% of moisture and yielded 29.0% (on dry weight) of a solid brown fat when extracted with light petroleum (b. p. 40–60°) in a Bolton–Revis apparatus.

The crude fat had acid value 38 (corresponding to 13.6% free acid as lauric), saponification equivalent 236.0 and iodine value 13.0; 89.0 g. was neutralized by shaking its ether solution several times with 10% aqueous potassium carbonate; the neutral fat recovered (73 g.) had saponification equivalent 233.0 and iodine value 10.1, while the acid fraction (16 g.) recovered from the potassium carbonate washings had saponification equivalent 243.9 and iodine value 25.9.

The free fatty acids are thus more unsaturated than the neutral fat. This phenomenon has been observed by Atherton and Meara[5] in the fats of *Virola surinamensis* and *Pycnanthus Kombo*, which are rich in myristic acid. Unfortunately, for reasons given below, we were unable to complete further examination of this fraction.

The neutral fat (52.1 g.) was saponified with alcoholic caustic soda. Extraction of the soap solution three times with light petroleum removed 0.72 g. of unsaponifiable matter of iodine value 81.5. It was later found that this treat-

(1) Puntambekar, *J. Indian Chem. Soc.*, **15**, 19 (1938).
(2) Gunde and Hilditch, *J. Chem. Soc.*, 1610 (1938).
(3) Trimen, "Handbook of the Flora of Ceylon," Vol. III, 450 (1895).
(4) Alston, *ibid.*, Vol. VI, 248 (1931).
(5) Atherton and Meara, *J. Soc. Chem. Ind.*, **63**, 353 (1939).