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CITRUS CAROTENOIDS—VI.

CAROTENOID PIGMENTS IN THE FLAVEDO OF SINTON CITRANGEQUAT

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Abstract—The flavedo of the fruit of the trigeneric hybrid Sinton citrangequat contains new and unusual carotenoid ketones (apo-carotenones). These pigments are unique in the carotenoid series in that they contain the terminal methyl ketone group in the side chain. Thus far, the methyl ketone carotenoids with nonaeneone and decaeneone chromophores have been isolated and characterized. They are sintaxanthin, citranaxanthin and reticulataxanthin. A minor carotenoid ketone, probably 3-OH-sintaxanthin, was also present. A methyl ketone carotenoid containing an in-chain hydroxyl group, 8′-OH-7′8′-dihydrocitranaxanthin, was also isolated. The remainder of the carbonyl carotenoids consisted of β-apo-10′-carotenal, β-apo-8′carotenal, β-citraurin, and probably 3-OH-β-apo-10′-carotenal. β-Zeacarotene was isolated for the first time from citrus. Neurosporene, γ-carotene and β-carotene in minor amounts were also detected. The rich red color of the flavedo is due mainly to the methyl ketone carotenoids.

INTRODUCTION

SINTON citrangequat[2] is a hybrid of the oval kumquat (*Fortunella margarita*) with Rusk citrange (*Poncirus trifoliata* × *Citrus sinensis*). Thus, it is a trigeneric hybrid of the genera *Fortunella*, *Poncirus*, and *Citrus*. The tree has deep orange to red fruit which is wholly or nearly seedless. The fruit of the oval kumquat is yellow–orange to light orange, whereas the Rusk citrange is slightly deeper orange. The fruit of *Poncirus trifoliata* grown in Indio, California, ranges from yellow to light orange. California oranges (*Citrus sinensis*) are usually orange. Neither the species nor bigeneric hybrid mentioned above approached the deep color of the fruit of the Sinton citrangequat.

To date, studies on the carotenoids of citrus fruits have been limited mainly to *Citrus*, such as *Citrus sinensis*,[3] *Citrus paradisi*,[4] and *Citrus reticulata*.[5] No detailed investigations of the carotenoids of *Citrus* hybrids have been carried out.

Initial studies[6–9] of the Sinton citrangequat indicated the presence of several unique apo-carotenones. Subsequently, a detailed examination of the carotenoid pattern in the flavedo of the fruit was undertaken with a view to fitting such findings into a biochemical sequence and explaining the syntheses of the compounds in nature.

[1] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[2] W. T. SWINGLE, In *The Citrus Industry* (Edited by H. J. WEBBER and L. D. BATCHELOR), Vol. I, p. 666. University of California Press, Berkeley and Los Angeles (1943).

[3] A. L. CURL and G. F. BAILEY, *J. Agr. Food Chem.* **2**, 685 (1954); A. L. CURL and G. F. BAILEY, *J. Agr. Food Chem.* **4**, 156 (1956); A. L. CURL and G. F. BAILEY, *J. Food Sci.* **26**, 442 (1961).

[4] A. L. CURL and G. F. BAILEY, *Food Res.* **22**, 63 (1957).

[5] A. L. CURL and G. F. BAILEY, *J. Agr. Food Chem.* **5**, 605 (1957).

[6] H. YOKOYAMA and M. J. WHITE, *J. Org. Chem.* **30**, 2481 (1965).

[7] H. YOKOYAMA and M. J. WHITE, *J. Org. Chem.* **30**, 2482 (1965).

[8] H. YOKOYAMA and M. J. WHITE, *J. Org. Chem.* **30**, 3994 (1965).

[9] H. YOKOYAMA and M. J. WHITE, *J. Org. Chem.* (In press).

RESULTS

The complex carotenoid mixture was initially separated into two fractions (Tables 1 and 2) by phase-partition between light petroleum and 90% methanol. The individual pigments were isolated for identification by chromatography of each phase on columns of different adsorbents.

TABLE 1. CHROMATOGRAPHIC SEPARATION OF THE EPIPHASIC CAROTENOIDS FROM THE FLAVEDO OF SINTON CITRANGEQUAT

Fraction*	Color	Required eluant† in light petroleum‡	Identity	Per cent of total carotenoids
1a	Yellow	3% E (0% A)	η-Carotene**	5·6
1b	Yellow	(1% A)	α-Carotene	0·2
1c-1	Yellow–orange	(2% A) (10% E)	β-Carotene	3·7
1c-2	Yellow	(20–30% E)	β-Zeacarotene	5·0
1c-3	Yellow	(40% E)	ζ-Carotene	1·3
1d	Yellow–orange	(5% A)	γ-Carotene	1·2
1e	Orange	(6% A)	Neurosporene	1·0
2	Yellow	(5% E)	Mutatochrome	1·7
3	Yellow–orange	(10% E)	β-Apo-10′-carotenal	1·9
4	Yellow–orange	(15% E)	Cryptoxanthin	0·1
5	Pink	(15–20% E)	β-Apo-8′-carotenal	0·6
6	Yellow	(15–20% E)	Unknown	6·0
7	Pink	(20–25% E)	Sintaxanthin	0·1
8	Purple	(40% E)	Citranaxanthin	10·8

* Adsorbent alumina (activity grade 3); pigments in order of increasing adsorption; Fraction 1 was rechromatographed on MgO–Hyflo-Super Cel (1:1); Fraction 1c was rechromatographed on alumina (activity grade 3).

† A=acetone; E=diethyl ether; solvents used in elution of fractions on rechromatography are in parenthesis.

‡ B.p. 30–60°.

** Provisional identification.

TABLE 2. CHROMATOGRAPHIC SEPARATION OF THE HYPOPHASIC CAROTENOIDS FROM THE FLAVEDO OF SINTON CITRANGEQUAT

Fraction*	Color	Required eluant in light petroleum†	Identity	Per cent of total carotenoids
1	Yellow–orange	2% Acetone	Unknown‡	0·1
2a	Orange	2–5% Acetone (30–60% ether)	β-Citraurin	3·3
2b	Yellow	(60–90% ether)	8′-OH 7′,8′-Dihydro-citranaxanthin	1·1
3	Pink	5–8% Acetone	3-OH-Sintaxanthin§	1·5
4	Orange	10–12% Acetone	Zeaxanthin	0·6
5	Red–purple	12–15% Acetone	Reticulataxanthin	49·8
6	Yellow–orange	20% Acetone	8′-OH 7′,8′-Dihydro-reticulataxanthin§	1·3

* Adsorbent Microcel C; fraction 2 rechromatographed on deactivated alumina; pigments in order of increasing adsorption.

† Solvents used in elution of fractions on rechromatography are in parenthesis.

‡ Probably 3-OH-β-apo-10′-carotenal.

§ Provisional identification.

Adsorbed just above phytofluene and ahead of α-carotene on a column of magnesium oxide was a component with an absorption spectrum similar to that of ζ-carotene. An authentic specimen of ζ-carotene isolated from tomatoes[10] was, however, easily separable from the unknown carotene on a column of magnesium oxide. Morevover, both the unknown and ζ-carotene occur together in the flavedo. The unknown carotene is probably similar to the η-carotene detected in *Lonicera* berries by Goodwin.[11] No direct comparison with η-carotene was attempted. A colorless material cochromatographing with the unknown carotene, precluded isolation of enough crystals for structural elucidation.

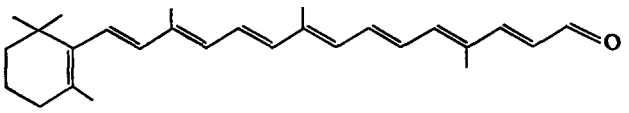
α-Carotene was identified from its absorption spectrum and mixed thin-layer chromatogram with authentic α-carotene isolated from carrot root.[12] The identity of β-carotene was proved by cochromatography on thin-layer plates and comparison of visible spectrum with that of synthetic β-carotene.

β-Zeacarotene was isolated for the first time from citrus fruit. The pigment was identified by comparison with authentic β-zeacarotene isolated from yellow corn[13, 14] by visible spectra and cochromatography. Only one form of β-zeacarotene was detected in the flavedo. This finding is in line with the observation of Simpson and Goodwin.[14]

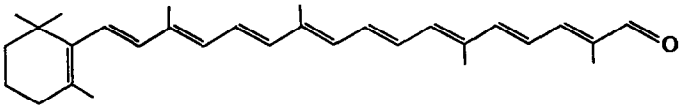
On a column of MgO two minor constituents adsorbed above β-carotene and mutatochrome (Table 1) were shown to be γ-carotene and neurosporene by the identities of their visible spectral and chromatographic properties with those of authentic samples.

A little mutatochrome was present. It was identified by comparison of the visible spectral and chromatographic properties with those of synthetic mutatochrome. The compound also gave a positive epoxide test.

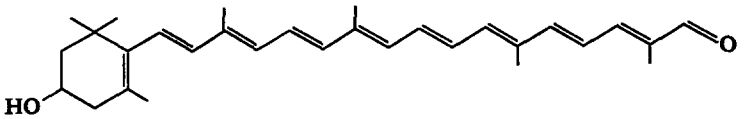
Three apo-carotenals were isolated and identified. These were β-apo-10'-carotenal (I), β-apo-8'-carotenal (II) (Table 1), and β-citraurin (III) (Table 2). Thommen[15] previously



(I) β-Apo-10'-carotenal



(II) β-Apo-8'-carotenal



(III) β-Citraurin

[10] J. W. PORTER and F. P. ZSCHEILE, *Arch. Biochem.* **10**, 537 (1946).
[11] T. W. GOODWIN, *Biochem. J.* **51**, 458 (1952).
[12] G. MACKINNEY, *J. Biol. Chem.* **111**, 75 (1935).
[13] E. N. PETZOLD, F. W. QUACKENBUSH and M. MCQUISTAN, *Arch. Biochem. Biophys.* **82**, 117 (1959).
[14] K. L. SIMPSON and T. W. GOODWIN, *Phytochem.* **4**, 193 (1965).
[15] H. THOMMEN, *Naturwissenschaften* **49**, 517 (1962).

reported these apo-carotenals in the juice and peel of fresh oranges. The identities of β-apo-10′-carotenal (I) and β-apo-8′-carotenal (II) were established by direct comparison with synthetic samples. The natural and synthetic samples could not be separated by thin-layer

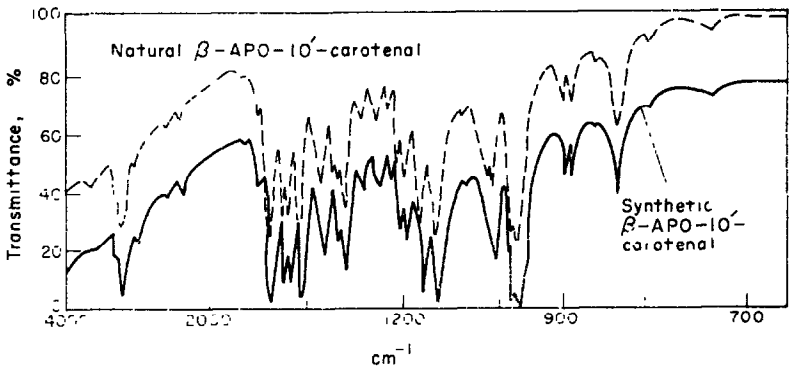


FIG. 1. INFRA-RED SPECTRA OF NATURAL AND SYNTHETIC β-APO-10′-CAROTENAL IN KBr.

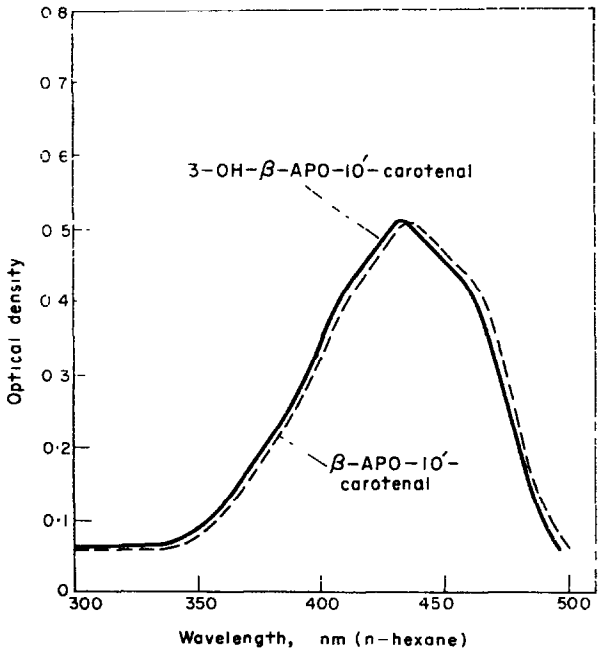


FIG. 2. THE ABSORPTION SPECTRA OF β-APO-10′-CAROTENAL AND CAROTENOID TENTATIVELY IDENTIFIED AS 3-OH-β-APO-10′-CAROTENAL.

chromatography, and both samples showed the same hypsochromic shift in absorption maxima on reduction with $NaBH_4$. Subsequently, from a larger amount of peels, crystalline β-apo-10′-carotenal was isolated, and a direct comparison of the i.r. spectra (Fig. 1) of the natural and synthetic samples was made. These identity criteria prove the structure (I) for the apo-carotenal.

By working up a still larger amount of peel, pure β-citraurin was isolated and crystallized from peroxide-free ether and *n*-hexane. The melting point of β-citraurin and its oxime derivative corresponded to those reported in the literature. By chromatographic and visible spectral criteria the isolated pigment was identical to β-citraurin isolated from the alkali cleavage of reticulataxanthin.[7]

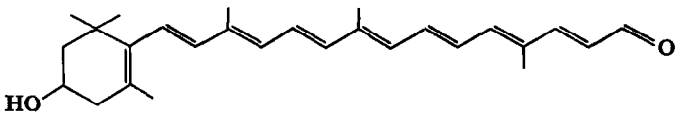
An apo-carotenal with a chromophore which corresponds to that of β-apo-10′-carotenal (see Fig. 2) (Table 2) was isolated. On reduction of the pigment with $NaBH_4$ a hypsochromic shift was observed, and the reduced visible spectrum was similar to that of the β-apo-10′-carotenol. The reduced compound resisted acid chloroform treatment. The partition ratio (Table 3) showed that the pigment is more polar than β-apo-10′-carotenal; a hydroxyl group

TABLE 3. RELATIVE POLARITIES OF CARBONYL CAROTENOIDS

Carotenoid	Observed polarity*
Citranaxanthin	1·06
Reticulataxanthin	2·10
Sintaxanthin	1·10
3-OH-sintaxanthin	2·14
β-Apo-8′-carotenal	0·94
β-Citraurin	1·95
β-Apo-10′-carotenal	1·27
Unknown from zone 1 (hypophase)	2·27

* Determined according to the method described by Krinsky.[33]

is probably present. We tentatively suggest structure (IV) for this pigment. Owing to lack of material no direct comparison with an authentic sample has so far been carried out, but this structure is plausible in view of the similarity of the pigment to β-apo-10′-carotenal. Natural hydroxylated carotenoids usually have the OH group in the 3-position.



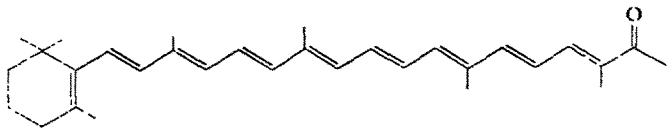
(IV) 3-Hydroxy-β-apo-10′-carotenal

The identity of cryptoxanthin (Table 1) was established by direct comparison with a sample obtained from egg yolk.[16] By partition ratio, visible spectral, and mixed chromatogram criteria the isolated sample was identical to authentic cryptoxanthin.

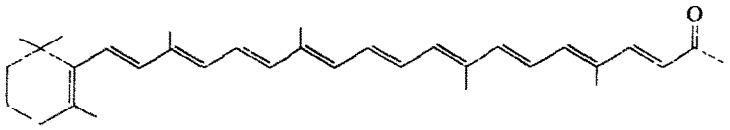
An unknown pigment with a chromophore (λ_{max} in *n*-hexane 403, 424 and 451 nm) similar to β-zeacarotene was isolated. Quantitative partition test indicated a polarity similar to that of cryptoxanthin. On acid chloroform treatment of the unknown pigment, no bathochromic shift in the absorption maxima could be detected, indicating the absence of an allylic OH group. The pigment gave a negative epoxide test. Structural studies are currently being carried out.

Two new, unique apo-carotenones were isolated in the crystalline state (Table 1). We named these carotenoid ketones sintaxanthin (V)[8] and citranaxanthin (VI).[6] The structure of

[16] A. E. GILLAM and I. M. HEILBRON, *Biochem. J.* **29**, 1064 (1935).



(V) Sintaxanthin



(VI) Citranaxanthin

sintaxanthin (V) includes a nonaeneone chromophore. Citranaxanthin (VI) contains a decaeneone chromophore. The visible spectra of sintaxanthin (Fig. 3) and citranaxanthin (Fig. 4) conform to those of nonaeneone and decaeneone chromophores respectively. The complete elucidations of structure have been reported elsewhere.[6, 8]

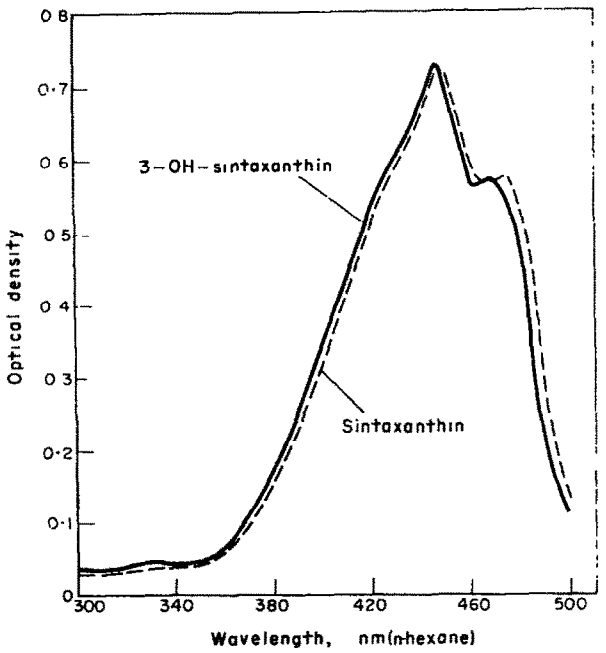


FIG. 3. THE ABSORPTION SPECTRA OF SINTAXANTHIN AND CAROTENOID TENTATIVELY IDENTIFIED AS 3-OH-SINTAXANTHIN.

A carotenoid constituent with a chromophore (Fig. 3) similar to sintaxanthin was also isolated (Fraction 3, Table 2). A comparison of the partition ratios (Table 3) of the isolated pigment and sintaxanthin suggested that the former contains a hydroxyl group. On reduction of the pigment with $NaBH_4$, a hypsochromic shift with a resultant fine structure was observed. The spectrum corresponded closely to that of reduced sintaxanthin. The bathochromic shift on acid chloroform treatment of the reduced compound was insufficient to indicate dehydration

of allylic OH group on the ring. Due to insufficient sample no further structural study has been carried out. We tentatively suggest structure (VII) for this pigment. Owing to lack of material no direct comparison with an authentic sample has so far been conducted. This structure (VII) is plausible in view of the similarity of the isolated ketone and sintaxanthin.

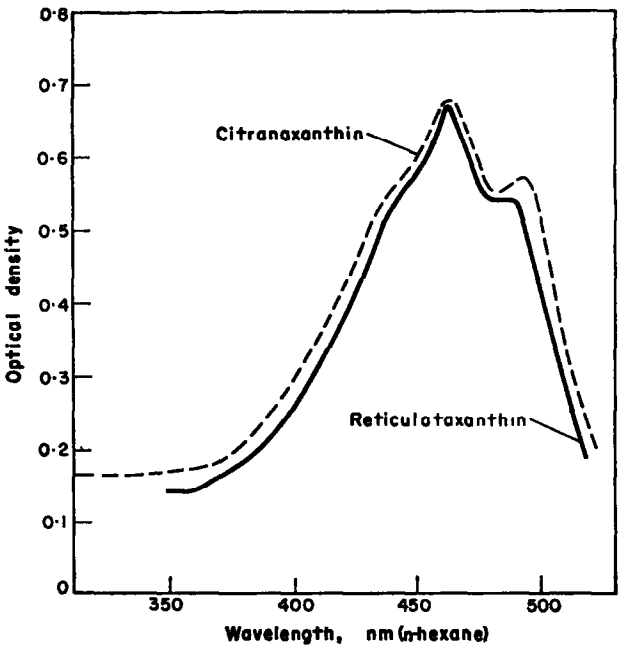
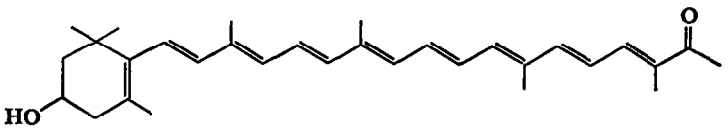


FIG. 4. THE ABSORPTION SPECTRA OF CITRANAXANTHIN AND RETICULATAXANTHIN.

The main pigment (Table 2) in the flavedo of Sinton citrangequat was reticulataxanthin (VIII).[17] The rich red color of the flavedo examined is due mainly to reticulataxanthin (Fig. 4). Pure reticulataxanthin was isolated in the crystalline state. Its identity was proved by comparison with an authentic sample of reticulataxanthin isolated from *Minneola tangor*.[7] As shown in structure (VIII), reticulataxanthin is 3-OH-citranaxanthin.



(VII) 3-Hydroxysintaxanthin

A new carotenoid, 8′-OH-7′8′-dihydrocitranaxanthin (IX)[9] was isolated. The visible spectrum in *n*-hexane is given in Fig. 5. On HCl–$CHCl_3$ treatment the pigment (Fig. 6) dehydrated readily to give citranaxanthin (VI). Elucidation of structure of the pigment is reported elsewhere.[9]

[17] A. L. CURL, *J. Food Sci.* **27**, 537 (1962).

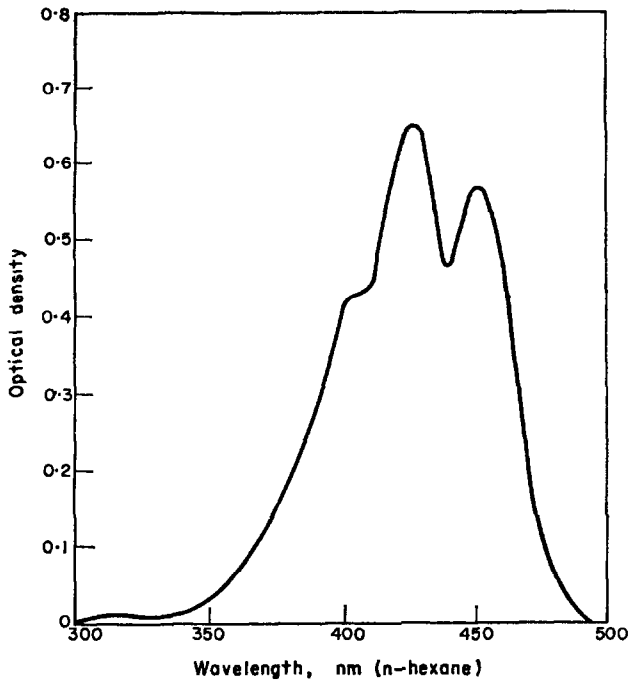


FIG. 5. THE ABSORPTION SPECTRUM OF 8′-HYDROXY-7′,8′-DIHYDROCITRANAXANTHIN.

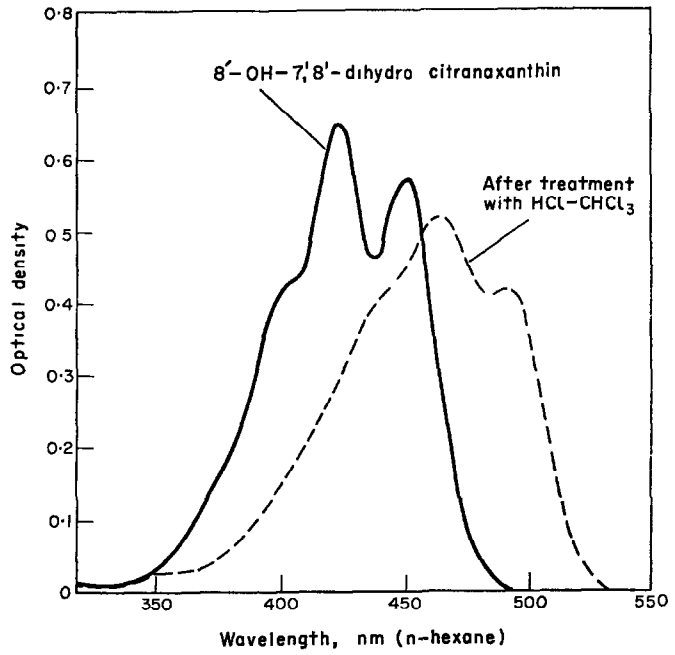
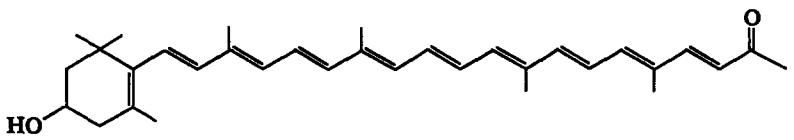
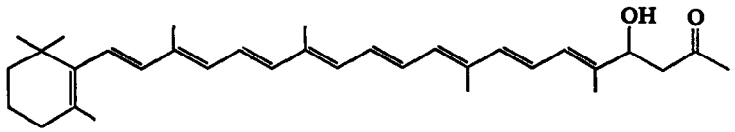


FIG. 6. THE ABSORPTION SPECTRA OF 8′-HYDROXY-7′,8′-DIHYDROCITRANAXANTHIN BEFORE AND AFTER TREATMENT WITH ACID CHLOROFORM.

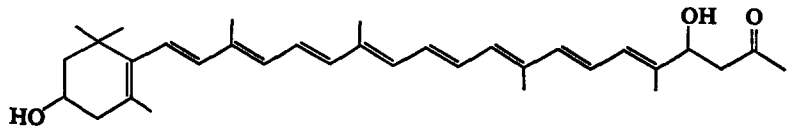


(VIII) Reticulataxanthin



(IX) 8′-Hydroxy-7′8′-dihydrocitranaxanthin

A polar constituent with absorption maxima (Fig. 7) similar to 8′-OH-7′,8′-dihydrocitranaxanthin was detected (Fraction 6, Table 2). Only a small quantity was isolated so a complete structural study was not attempted. On treatment with HCl–$CHCl_3$ the compound (Fig. 8) dehydrated readily to give reticulataxanthin (VIII). On the basis of this observation we suggest structure (X). Due to lack of material no direct comparison has so far been carried out.



(X) 8′-Hydroxy-7′8′-dihydroreticulataxanthin

Zeaxanthin was isolated and identified by the comparison of the visible spectrum and chromatographic properties with those of zeaxanthin from yellow corn.[18]

DISCUSSION

New and unusual apo-carotenones are synthesized in the flavedo of the fruit of Sinton citrangequat. These pigments contain carbon skeletons possessing fewer than forty carbon atoms and are unique among the carotenoid series in that they contain the terminal methyl ketone grouping in the side chain. So far methyl ketone carotenoids with nonaeneone and decaeneone chromophores have been isolated and characterized (V), (VI), (VIII) and (IX). These apo-carotenones are not species-specific but appear to be restricted to the family Rutaceae.[19] Previously kryptocapsin[20] and capsanthin,[21] carotenoids with a decaeneone chromophore but with a terminal pentane ring, were isolated from the fruit of *Capsicum annum*.

Reticulataxanthin was first isolated as a minor constituent from the peel of the fruit of *Citrus sinensis* by Curl and Bailey.[3] Subsequently, a richer source of the pigment was found

[18] L. Zechmeister and L. Cholnoky, *Ann. Chem.* **481**, 42 (1930).
[19] H. Yokoyama and M. J. White (Unpublished data).
[20] L. Cholnoky, K. Györgyfy, E. Nagy and M. Pánczél, *Acta Chim. Acad. Sci. Hung.* **6**, 143 (1955); L. Cholnoky, J. Szabolcs, R. D. G. Cooper and B. C. L. Weedon, *Tetrahedron Letters* **19**, 1257 (1963).
[21] L. Zechmeister and L. Cholnoky, *Ann Chem.* **543**, 248 (1940); M. S. Barber, L. M. Jackman, C. K. Warren and B. C. L. Weedon, *Proc. Chem. Soc.* 19 (1960); M. S. Barber, L. M. Jackman, C. K. Warren and B. C. L. Weedon, *J. Chem. Soc.* 4019 (1961); R. D. G. Cooper, L. M. Jackman and B. C. L. Weedon, *Proc. Chem. Soc.* 215 (1962).

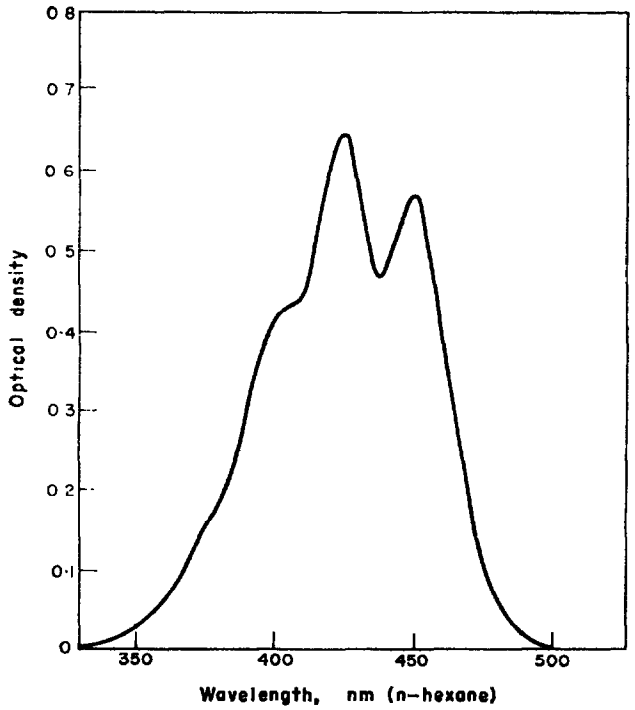


FIG. 7. THE ABSORPTION SPECTRUM OF THE CAROTENOID TENTATIVELY IDENTIFIED AS 8′-HYDROXY-7′,8′-DIHYDRORETICULATAXANTHIN.

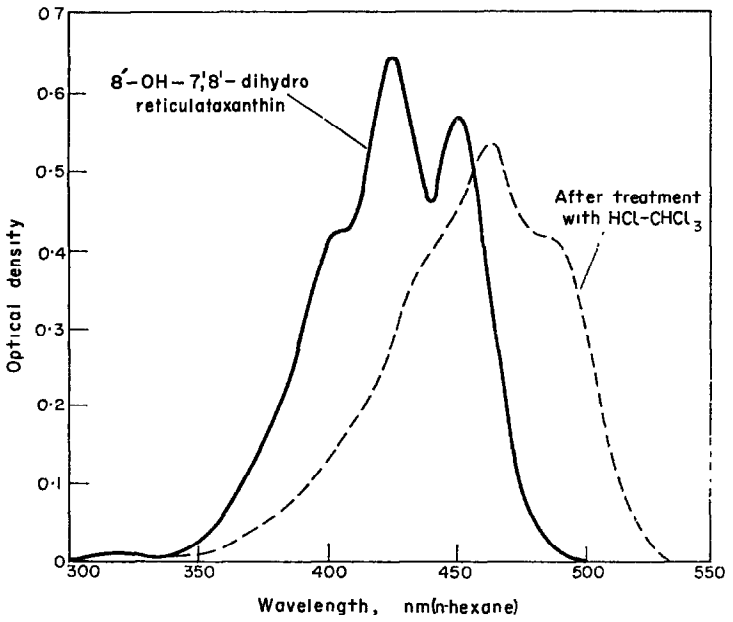


FIG. 8. THE ABSORPTION SPECTRA OF THE CAROTENOID TENTATIVELY IDENTIFIED AS 8′-HYDROXY-7′,8′-DIHYDRORETICULATAXANTHIN BEFORE AND AFTER TREATMENT WITH ACID CHLOROFORM.

in the peel of the *Citrus* hybrid, *Minneola tangor* (*Citrus reticulata* × *Citrus sinensis*).[7] Reticulataxanthin constitutes over 49 per cent of the total carotenoids in the trigeneric hybrid, Sinton citrangequat (Table 2.) Reticulataxanthin and other methyl ketone carotenoids are present in minor amounts or could not be detected in the parent species[19] of Sinton citrangequat, *Citrus sinensis*, *Poncirus trifoliata*, or *Fortunella margarita*, or in Rusk citrange (*Citrus sinensis* × *Poncirus trifoliata*).

In the case of *Minneola tangor* also the methyl ketone carotenoids emerge as major carotenoids only on hybridization. Reticulataxanthin is a minor constituent in *Citrus reticulata*,[5, 17] and in *Citrus sinensis*.[3] The ability to synthesize the methyl ketone carotenoids appears to increase with hybridization. The effect of hybridization will be discussed in more detail in a later paper.

The isolation of β-apo-10′-carotenal, β-apo-8′-carotenal, and β-citraurin suggests that the degradative transformations of the types advanced by Glover and Redfearn[22] and others[23, 24] may operate in the flavedo. Thus, it seems possible that β-apo-10′-carotenal and β-apo-8′-carotenal are natural degradation products of β-carotene, and β-citraurin of zeaxanthin.

The biogenesis of the methyl ketone carotenoids remains to be investigated.

The flavedo of Sinton citrangequat synthesizes neurosporene, γ-carotene, β-zeacarotene, and bicyclic β-carotene. This carotene complex is typical of yeast and suggests a biosynthetic sequence similar to that in *Rhodotorula glutinus*.[25]

The occurrence of an η-carotene-like compound in the fruit collected in 1964 is of interest. On prolonged storage of the fruit the unknown carotene disappeared, and ζ-carotene accumulated.[19] No η-carotene-like compound was found in the fruit collected in 1965.

EXPERIMENTAL

Fruits

The fruits used in the study on the carotenoid composition were collected in 1964 at the USDA Date and Citrus Experiment Station, Indio, California. Additional lots used in identification studies were obtained in 1964 and 1965 from the USDA Crops Research Division, Weslaco, Texas, and Orlando, Florida. These fruits were collected from January to February when in the most highly pigmented stage. The peels ranged in color from deep orange to dark red; those collected in 1964 were richer in color.

Pigment Extraction

The peel was separated from the endocarp by hand. The carotenoid pigments were extracted from the peels successively with acetone and methanol in the Waring blendor. The combined extract was diluted with an equal volume of diethyl ether (previously passed through a column of alumina to remove the peroxides[26]) and sufficient saturated NaCl solution added to form two layers. The ether layer was combined with an equal volume of 10% KOH–ethanol, covered with nitrogen, and kept for 2 hr at room temperature with occasional shaking. The mixture was washed free of alkali, dried over anhydrous $MgSO_4$,

[22] J. Glover and G. R. Redfearn, *Biochem. J.* **58**, XV (1954).
[23] J. A. Blair, In *Carotine and Carotinoide*, Dr. Dietrich Steinkopff Verlag, Darmstadt (1963).
[24] O. Isler, R. Rüegg and P. Schudel, In *Recent Progress in the Chemistry of Natural and Synthetic Coloring Matters and Related Fields* (Edited by T. S. Gore, B. S. Joshi, S. V. Sunthanker and B. D. Telak), p. 39. Academic Press, New York (1962).
[25] K. L. Simpson, T. O. M. Nakayama and C. O. Chichester, *J. Bacteriol.* **88**, 1688 (1964).
[26] W. Dasler and C. D. Bauer, *Ind. Eng. Chem., Anal. Ed.* **18**, 52 (1946).

filtered, and evaporated *in vacuo*. The nonsaponifiable matter was taken up in light petroleum (b.p. 30–60°) and methanol in the usual manner for later phase-partition separation.

Chromatographic Separation

In the initial studies on the composition of carotenoids in the flavedo, the pigment mixture was phase-partitioned between light petroleum and 90% methanol.

The epiphasic pigment mixture was chromatographed on a column of alumina (activity grade 3). The column was developed with light petroleum containing increasing amounts of peroxide-free ether. The front-running hydrocarbon fraction was rechromatographed on a column of magnesium oxide–Hyflo Supercel (1:1, w/w). The separation of β-carotene, ζ-carotene, and β-zeacarotene was difficult but good resolution was obtained on a column of alumina (activity grade 1).

The hypophasic carotenoids were chromatographed initially on a column of Microcel C. The column was developed and eluted (step by step gradient elution) with light petroleum containing increasing amounts of acetone. Partially resolved carotenoids were further separated on deactivated alumina (activity grade 3) and eluted with light petroleum containing increasing amounts of peroxide-free ether. In later separation work, a Craig apparatus was used according to the method employed by Curl[27] except that the tubes were initially flushed with N_2 and kept under nearly constant N_2 atmosphere.

Cochromatography Tests

Thin-layer chromatography was employed in the comparison of chromatographic properties. The thin-layer plates were prepared in the usual manner.[28] As a safeguard, the plates were developed under subdued light and CO_2 atmosphere.

The adsorbent used for separation of carotenes was calcium hydroxide–silica gel G (6:1), and the solvent system consisted of light petroleum–benzene (98:2)[29] unless otherwise stated. For separation of methyl ketones and aldehydes the same layer as described above and benzene or a solvent mixture of light petroleum–benzene (1:1) was used.[30] For the polar xanthophylls, reversed-phase thin-layer chromatography was carried out as described by Randerath.[31]

Quantitative Determination

The usual method used has been described by Davies.[32]

Identification of Pigments

The individual pigments were identified on the bases of comparison of chromatographic movements and visible spectra with those of authentic samples. The i.r. spectra were compared whenever possible. In certain cases mixture melting points were determined for crystalline pigments. Partition ratios were also determined in certain cases.[33, 34] Cases where

[27] A. L. CURL, *J. Agr. Food Chem.* **1**, 456 (1953); A. L. CURL, *J. Agr. Food Chem.* **8**, 356 (1960).
[28] E. STAHL (Ed.), *Thin-Layer Chromatography*. Academic Press, New York (1965).
[29] H. BOLLIGER, In *Thin-Layer Chromatography* (Edited by E. STAHL). Academic Press, New York (1965).
[30] A. WINTERSTEIN, A. STUDER and R. RÜEGG, *Chem. Ber.* **93**, 295 (1960); A. WINTERSTEIN and B. HEGEDÜS, *Chimia* **14**, 18 (1960).
[31] K. RANDERATH, *Thin-Layer Chromatography*. Academic Press, New York (1963).
[32] B. H. DAVIES, *Phytochem.* **1**, 25 (1962); B. H. DAVIES, In *Chemistry and Biochemistry of Plant Pigments* (Edited by T. W. GOODWIN). Academic Press, New York (1965).
[33] N. I. KRINSKY, *Anal. Biochem.* **6**, 293 (1963).
[34] F. J. PETRACEK and L. ZECHMEISTER, *Anal. Chem.* **28**, 1484 (1956).

ambiguity exists because authentic samples were not available for comparison or because the small amount isolated precluded detailed structural studies are noted in the results. Iodine catalysis was carried out as described previously.[8]

Epiphasic Carotenoids

η-Carotene. The epiphasic carotenoid mixture was chromatographed on a column of alumina (activity grade 3) and developed with light petroleum containing increasing amounts of diethyl ether (3–40 per cent). Seven zones were separated. Zone 1 was rechromatographed on MgO–Hyflo Super Cel (1:1, w/w). Zone 1a exhibited absorption maxima at 376, 397, and 422 nm in light petroleum. On isomerization with iodine and light the abs. max. shifted down to λ_{max} 374, 395 and 420 nm. Upon cochromatography on thin-layer plates (developed with light petroleum–benzene (98:2)) with authentic ζ-carotene isolated from tomatoes,[10] two distinct yellow zones appeared. ζ-Carotene was more strongly adsorbed. Column chromatography on MgO (with light petroleum as developing solvent) of a mixture of ζ-carotene and the isolated yellow pigment showed separation into two distinct zones with ζ-carotene being more strongly adsorbed.

α-Carotene. Zone 1b exhibited absorption spectrum (423, 446, and 475 nm in light petroleum) undistinguishable from that of α-carotene from carrots.[12] Cochromatography tests on thin-layer plates revealed a single zone (developing agent light petroleum–benzene (98:2)).

β-Carotene. The pigments in zone 1c were rechromatographed on a column of alumina (activity grade 1) and developed with light petroleum containing increasing amounts of diethyl ether (10–50 per cent). Three zones (1c-1, 1c-2, and 1c-3) formed. Zone 1c–1 exhibited absorption maxima at 425, 448, and 478 nm in light petroleum. The visible spectrum was superimposable on that of synthetic β-carotene. A mixed, thin-layer chromatogram with synthetic β-carotene revealed a single zone (solvent system light petroleum–benzene).

β-Zeacarotene. Zone 1c-2 from rechromatography of zone 1c as described above exhibited absorption maxima at 404 (sh), 425, and 452 nm in light petroleum. The visible spectral curve of the isolated pigment was indistinguishable from that of β-zeacarotene from yellow corn. Mixed thin-layer chromatogram with β-zeacarotene isolated from yellow corn revealed a single zone (solvent system light petroleum–benzene (97:3)).

ζ-Carotene. Zone 1c-3 from above had absorption maxima at 377, 398, and 423 nm in light petroleum. The u.v. spectral curve was identical to that of ζ-carotene from tomatoes.[10] Mixed thin-layer chromatogram with authentic sample showed a single zone (developing agent light petroleum–benzene (97:3)).

γ-Carotene. The isolated pigment (zone 1d) exhibited absorption maxima at 438, 462, and 493 nm in light petroleum and was shown to be γ-carotene by the identity of its visible spectral and chromatographic criteria with those of γ-carotene from carrots.[35] Cochromatography tests on thin-layer plates revealed one zone (solvent system light petroleum–benzene (97:3)).

Neurosporene. Zone 1c from above exhibited absorption maxima at 414, 438, and 468 nm in *n*-hexane. It eluted with 60 per cent diethyl ether in light petroleum. The visible spectrum of the isolated pigment was identical to that of neurosporene from *Rhodotorula glutinis.*[25] A mixed chromatogram on a column of magnesia developed with 4 per cent acetone in light petroleum revealed a single zone.

[35] R. KUHN and H. BROCKMANN, *Chem. Ber.* **66**, 407 (1933).

Mutatochrome. Zone 2 was identical to synthetic mutatochrome.[36] The isolated pigment exhibited absorption maxima at 408, 420, and 447 nm in light petroleum. The visible spectrum exhibited pronounced fine structure. Mixed column chromatogram on alumina (activity grade 3, solvent system 10 per cent diethyl ether in light petroleum) revealed a single zone. On treatment with ethanolic-HCl the isolated pigment turned light blue.

β-Apo-10′-carotenal (I). Zone 3 had absorption maxima at 432 and 458 (sh) nm in light petroleum. The visible spectrum was superimposable on that of the synthetic β-apo-10′-carotenal. A larger amount of peel was worked up, and about 8 mg of the pigment was obtained in the crystalline state from *n*-hexane. Direct comparison of the i.r. spectra of the natural and synthetic samples (Fig. 1) indicates that they are identical. Mixed thin-layer chromatogram revealed a single zone [(solvent system light petroleum–benzene) (1 : 1)].

Cryptoxanthin. Zone 4 had absorption maxima at 425 (sh), 446, and 475 nm in *n*-hexane. The visible spectrum was identical to that of cryptoxanthin from egg yolk.[16] Partition ratio found: *n*-hexane– 95% methanol, 85:15. Mixed thin-layer chromatogram with benzene as solvent system revealed one zone.

β-Apo-8′-carotenal (II). Zone 5 in light petroleum exhibited λ_{max} 451 and 478 nm, in ethanol 463 nm. The visible spectra in the two solvents were indistinguishable from those of synthetic β-apo-8′-carotenal. Mixed thin-layer chromatography showed a single zone. On reduction with $NaBH_4$ in the usual manner,[6] the same hypsochromic shift in λ_{max} was observed, and the reduced visible spectra (λ_{max} in light petroleum 404, 425, and 451 nm) were identical.

Unknown pigment from zone 6. The visible spectrum [λ_{max} in *n*-hexane 403 (sh), 424 and 451 nm] of the unknown pigment exhibited a fine structure and corresponded closely to that of β-zeacarotene. Quantitative partition test (hexane–95% methanol, 81:19) indicated a more polar compound than β-zeacarotene. The unknown pigment gave a negative epoxide test. On treatment with chloroformic hydrogen chloride, the unknown pigment resisted dehydration. Further studies on the structure of this unknown pigment are being carried out.

Sintaxanthin (V). Zone 7 had λ_{max} in light petroleum 428 (sh), 448, and 475 nm. The details of the properties and elucidation of structure have been reported previously.[8]

Citranaxanthin (VI). Zone 8 yielded a carbonyl carotenoid with λ_{max} in *n*-hexane, 463 and 495 nm. The full description of this pigment has been reported previously.[7]

Hypophasic Carotenoids

Unknown pigment from zone 1. The isolated had λ_{max} in *n*-hexane 431 and 456 (sh) nm. The visible spectrum corresponds closely to that of β-apo-10′-carotenal. Comparison of the relative polarities of some carbonyl carotenoids as determined by the method of Krinsky[33] is given in Table 3. The polarity of β-apo-10′-carotenal is 1·27 and that of the unknown is 2·28. Thus, the relative polarity of the unknown pigment is 1·01 which suggests the presence of a non-allylic hydroxyl group[33] on the ring, probably the 3 position. Reduction of the unknown pigment with $NaBH_4$ in the usual manner[6] gave a product which exhibited a hypsochromic shift in its absorption maxima. The resulting visible spectrum had λ_{max} in *n*-hexane 380 (sh), 398, and 422 nm.

β-Citraurin (III). Zone 2 was rechromatographed on deactivated alumina and developed with light petroleum containing increasing amounts of diethyl ether (30–90 per cent). Two zones (2a and 2b) were separated. Zone 2a had λ_{max} in *n*-hexane, 450 and 476 nm. The visible

[36] P. KARRER and E. JUCKER, *Helv. Chim. Acta* **28**, 427 (1945).

spectrum showed complete agreement with β-citraurin from alkali cleavage of reticulataxanthin.[7] A larger amount of peel was worked up and a small amount (about 5 mg) of the pigment was isolated in the crystalline state, m.p. β-citraurin isolated from peel 145–146°; β-citraurin isolated from degradation of reticulataxanthin 145–146°; lit.[37] 147°. The oxime prepared in the usual manner[6] had m.p. 187–188°, lit.[38] m.p. 188°.

8′-OH-7′8′-Dihydrocitranaxanthin (IX). Zone 2b from above had λ_{max} in *n*-hexane 402, 423, and 449 nm (Fig. 5). The complete structural study of this compound has been reported previously.[9]

3-OH-Sintaxanthin (VII). Zone 3 had λ_{max} in *n*-hexane 426 (sh), 446, and 474 nm. The visible spectrum corresponded closely to that of sintaxanthin (V). The relative polarity of 2·14 (Table 3) suggests a non-allylic hydroxyl in the 3 position on the ring. On reduction with $NaBH_4$ a hypsochromic shift in the absorption maxima was observed. The λ_{max} in *n*-hexane 403, 424, and 454 nm correspond closely to those of sintaxanthol.[8] On HCl–$CHCl_3$ treatment a bathochromic shift in the λ_{max} of 26 nm was observed.

Zeaxanthin. Zone 4 exhibited λ_{max} in *n*-hexane 425, 450, and 480 nm. The visible spectrum was indistinguishable from that of zeaxanthin from yellow corn. Mixed chromatography on a column of Microcel C showed only one zone. Partition ratio found: *n*-hexane–95% methanol 12:88.

Reticulataxanthin (VIII). The pigment from zone 5 was identical in every respect to reticulataxanthin isolated from *Minneola tangor*.[7]

8′-OH-7′8′-Dihydroreticulataxanthin (X). Zone 6 yielded a pigment with λ_{max} in *n*-hexane 401, 422, and 448 nm (Fig. 7). HCl–$CHCl_3$ treatment (Fig. 8) of the isolated pigment afforded a product which was identical to reticulataxanthin (VIII) in mixed thin-layer chromatography and visible spectra.

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Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

[37] P. KARRER and U. SOLMSSEN, *Helv. Chim. Acta* **20**, 682 (1937).
[38] L. ZECHMEISTER and L. V. CHOLNOKY, *Ann. Chem.* **530**, 291 (1937).