ORANGE CAROTENOIDS

Polyoxygen Carotenoids of Valencia Orange Juice

A. LAURENCE CURL and GLEN F. BAILEY

Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 6, Calif.

Because the carotenoid pigments of stored orange juice products may be a potential source of off flavors, the composition of the pigment mixture in fresh Valencia orange juice has been investigated. The saponified carotenoids were separated by countercurrent distribution into six fractions, three of which were previously reported to yield a total of eight constituents on chromatography. The other three fractions yielded 17 constituents, 10 of which were apparently different xanthophylls, the other seven stereoisomers. Five components were very probably antheraxanthin, mutatoxanthin, violaxanthin, auroxanthin, and zeaxanthin epoxide-furanoxide. Two other components were tentatively identified, while three were apparently previously undescribed. All 17 were apparently either xanthophyll epoxides or the corresponding furanoxides, which are, in general, less stable than the simpler carotenoids, especially in an acid medium.

THE APPLICATION of countercurrent distribution to the fractionation of the carotenoids of Valencia orange juice was reported in an earlier communication from this laboratory (*2*). By using two different solvent systems the saponified carotenoids can be divided into six fractions. In these runs the percentages of these six fractions in the total carotenoids were approximately as follows: I (hydrocarbons), 6%; II (monols), 13%; IIIA (diols), 22%; IIIB (monoether diols), 26%; IIIC (diether diols), 23%; and IIID (monoether polyols), 10%. The present report is concerned with an investigation of the components of fractions IIIB, IIIC, and IIID, which amounted to about 60% of the total carotenoids. In the absence of authentic samples for comparison, the components have been studied by means of countercurrent distribution, chromatography, spectrophotometry, and reaction with hydrochloric acid. On this basis a number of the components have been identified with a rather high degree of certainty, whereas several others appear to be previously undescribed carotenoids. The fractionation and identification of the saponified orange juice carotenoids as reported in a previous paper (*2*) and in the present one are summarized in Figure 1.

Experimental

The saponified lipide (unsaponifiable) fraction of Valencia orange juice was separated by means of countercurrent distribution in a 200-tube Craig apparatus, with a solvent system consisting of petroleum ether, benzene, and 87% methanol (1 to 1 to 1.15 by volume) into five fractions—I plus II (hydrocarbons plus monols), IIIA (diols), IIIB (monoether diols), IIIC (diether diols), and IIID (monoether polyols). Details of the materials, apparatus, and procedures, including chromatography, and identification of the constituents of fractions I, II, and IIIA were previously described (*2*).

Fractions IIIB, IIIC, and IIID, in benzene solution, were separately adsorbed on columns of magnesia (Westvaco No. 2642, Westvaco Chemical Division, Food Machinery & Chem. Corp., New York) plus diatomaceous earth (1 to 1 by volume), and fractionally eluted. A series of eluents of increasing strength was used, containing 3.5 to 50% of absolute ethanol in petroleum ether (boiling point 63° to 70° C.); solutions containing 10 to 20% of absolute ethanol in benzene were also sometimes used. The eluted fractions were evaporated in vacuo and dissolved in benzene or absolute ethanol, and spectral absorption curves were run in a Cary recording spectrophotometer, over the range 550 mμ to approximately 290 mμ.

Fractions IIIB, IIIC, and IIID on chromatography were resolved into five, six, and six components, respectively, all of which were different. Eight components had been previously found in fractions I, II, and IIIA (*2*), making a total of 25 in the carotenoid group (Figure 1). The wave lengths of the maxima and minima, together with relative absorbances at these points, are given in Table I. In each case the bands are numbered in the order that they were eluted from the column, IIIB1, IIIB2, IIIB3, etc. Data are also given for the cis-peaks (*20*), one of which was found for all components of fraction IIIB, and two for all components of fractions IIIC, and IIID where the cis-peaks fell above 290 mμ. Values for the cis-peaks of 0.10 or lower indicate only a slight inflection or low peak in the curves. Table I also includes the eluent used for each band, and the approximate percentages of each band in the three major fractions.

Hydrochloric Acid–Ether Test When an ethereal solution of a carotenoid containing one cyclic ether group in the molecule is shaken with concentrated hydrochloric acid, a pale or light-blue color forms in the lower layer; a deep-blue color indicates the presence of two cyclic ether groups (*4*). In order to compare the intensities of the colors formed with different fractions containing approximately equivalent amounts of carotenoids, and thus obtain an indication of the number of cyclic ether groups present in the molecule, a standardized procedure was adopted. From the absorbance (log I_0/I_x) of the highest peak on the spectrophotometer curve, the volume of solution was calculated which would be equivalent to 10 ml. of solution having an absorbance of 1.00 in a 1-cm. cell. This volume of solution was pipeted out, evaporated in vacuo, dissolved in 10 ml. of ether, 1 ml. of concentrated hydrochloric acid was added, and the mixture was shaken for about 30 seconds. In this test, the presence of peroxides in the ether caused the blue color to

turn purple and to fade much more rapidly than when the ether was low in peroxides; in the latter case, the blue color often persisted overnight.

The ether–hydrochloric acid tests was carried out on all fractions. None of the components obtained from fractions I, II, or IIIA gave any color. A pale or light-blue color was obtained from all components of fraction IIIB, suggesting the presence of one cyclic ether group. All components of fraction IIIC gave much stronger blue colors, suggesting presence of two cyclic ether groups. All components of fraction IIID gave blue colors of various degrees of intensity, indicating presence of cyclic ether groups.

Products Formed Carotenoid epoxides on treatment with dilute mineral acids, even with traces of hydrogen chloride as are present in old chloroform, are converted to the isomeric furanoxides, together with the corresponding desoxy compounds in which the ether oxygen has been removed (*3*). The products formed on hydrochloric acid treatment therefore are clues to the identity of a given fraction. This reaction was carried out with a number of the components of fractions IIIB, IIIC, and IIID, which were suspected of being epoxides. A portion of solution was evaporated to dryness in vacuo, and dissolved in 10 ml. of a solution of hydrochloric acid in methanol (1 volume of concentrated hydrochloric acid plus 9 volumes of methanol). After 1 or 2 minutes, an excess of potassium hydroxide in methanol was added (5 ml. of a 20% solution), and mixed well. The solution was transferred to a separatory funnel with 75 ml. of ether and 135 ml. of water and again mixed well. The ether layer was washed 6 times with water, 15 ml. of absolute ethanol was added, and evaporated in vacuo. The residue was dissolved in benzene and chromatographed as above. Cary spectrophotometer curves were run on all fractions—the absorption maxima for those fractions which were apparently pure or fairly pure are given in Table II.

Identification of Components of Fraction IIIB (Monoether Diols)

The following paragraphs are devoted to the probable or tentative identification of the various fractions; for convenience, the probable identity of the fraction is given at the beginning. When the fractions were apparently previously unidentified carotenoids, the proposed names are used.

Fraction IIIB1, Antheraxanthin The shape of the spectral absorption curve resembled that of zeaxanthin, but the maxima were 6 or 7 mμ lower, suggesting zeaxanthin epoxide (antheraxanthin). A mixed chromatogram of this fraction with lutein (which has similar absorption maxima) separated readily into two bands, the lower being lutein; with zeaxanthin, IIIB1 was the lower band. Antheraxanthin on treatment with hydrochloric acid was found to be converted to mutatoxanthin (zeaxanthin furanoxide) and zeaxanthin (*5*). IIIB1 was treated with hydrochloric acid in methanol and the resulting product on chromatography separated into five bands (Table II), the lowest apparently being a mixture. The reddish second band corresponded in absorption spectrum fairly well to zeaxanthin. The last two bands (4 and 5), which comprised the major part of the product, had similar absorption spectra that corresponded rather closely to those of fractions IIIB4 and IIIB5 (Table I). Fraction IIIB1–5 is probably mutatoxanthin. The spectral absorption curve of fraction IIIB1–4 had a somewhat higher cis-peak and slightly lower wave lengths of the maxima than IIIB1–5, and was probably a

Figure 1. Fractionation of saponified orange juice carotenoids by countercurrent distribution and chromatography

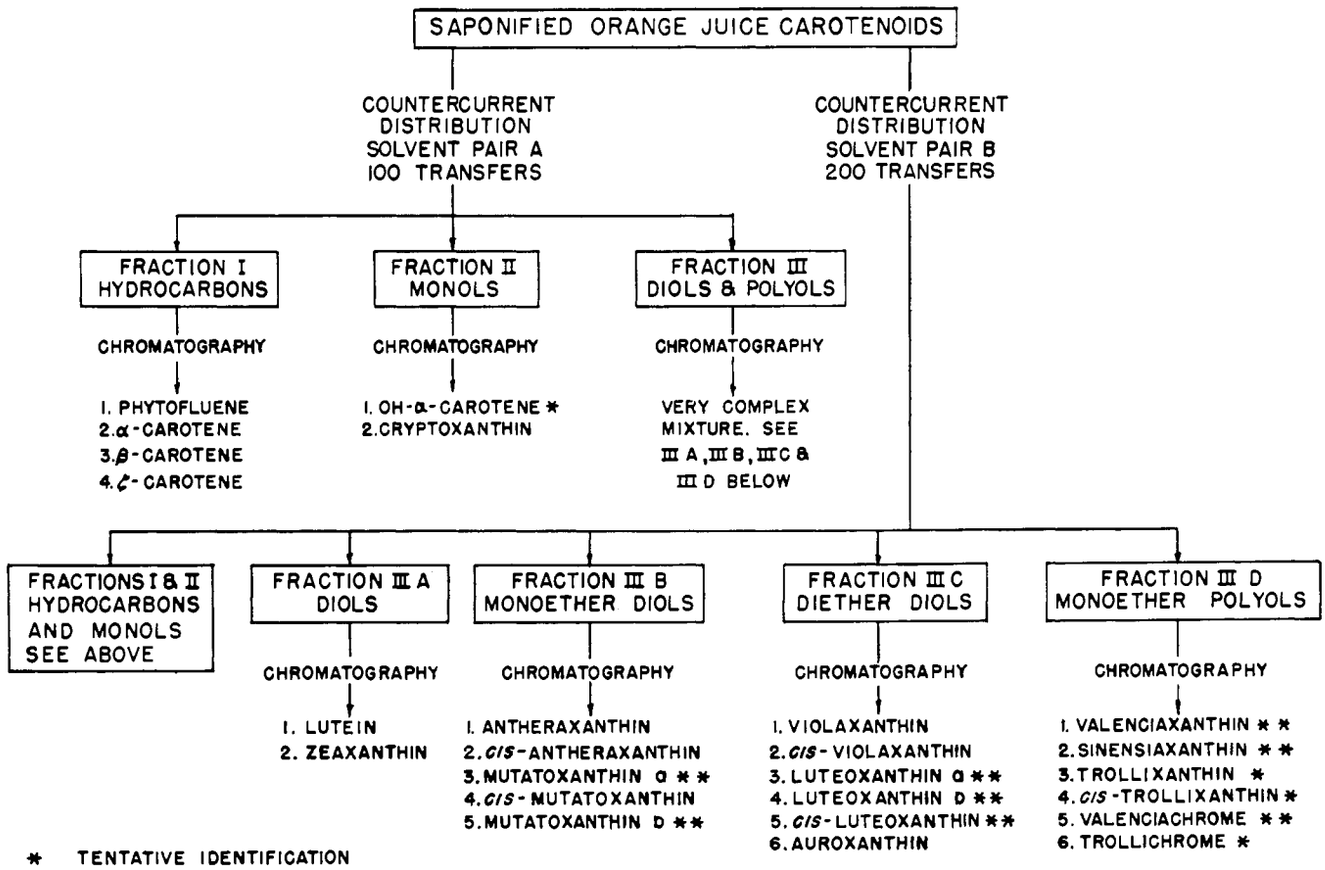

Table I. Polyoxygen Carotenoids Isolated from Valencia Orange Juice

Fraction No.	Probable Identity	Solvent[a]	Wave Length, mμ, of Maxima and Minima Relative Absorbancies in Parentheses							Eluent (% Ethanol in Pet. Ether)	Approx. % in Fraction IIIB, IIIC, or IIID
			Max.	Min.	Max.	Min.	Max.	cis-Peaks			
					IIIB (monoether diols)						
1	Antheraxanthin	B	485(0.83)	474(0.76)	455(1.00)	...	...	337(0.13)	...	3.5	46
2	*cis*-Antheraxanthin	B	482(0.92)	469(0.73)	453(1.00)	433(0.71)	428(0.71)	336(0.08)	...	5	30
3	Mutatoxanthin a[b]	B	464(0.89)	453(0.74)	437(1.00)	...	414(0.67)	320(0.08)	...	7	5
4	*cis*-Mutatoxanthin	B	463(0.84)	453(0.75)	436(1.00)	...	413(0.68)	322(0.15)	...	10	8
5	Mutatoxanthin b[b]	B	465(0.89)	453(0.73)	436(1.00)	...	414(0.67)	321(0.06)	...	15	12
					IIIC (diether diols)						
1	Violaxanthin	B	482(0.86)	468(0.67)	449(1.00)	430(0.71)	425(0.71)	335(0.17)	322(0.13)	3.5	11
2	*cis*-Violaxanthin	B	478(0.94)	465(0.51)	447(1.00)	432[0.57)	423(0.65)	335(0.09)	323(0.06)	5	61
3	Luteoxanthin a[b]	B	460(0.94)	447(0.50)	432(1.00)	416(0.56)	407(0.66)	319(0.09)	305(0.07)	7	4
4	Luteoxanthin b[b]	B	460(0.87)	447(0.56)	432(1.00)	416(0.63)	407(0.69)	320(0.15)	307(0.12)	7	2
5	*cis*-Luteoxanthin[b]	E	446(0.91)	434(0.56)	419(1.00)	405(0.60)	396(0.66)	313(0.09)	301(0.07)	10	16
6	Auroxanthin	E	427(0.97)	416(0.53)	402(1.00)	389(0.55)	381(0.63)	296(0.07)	...	50	5
					IIID (monoether polyols)						
1	Valenciaxanthin[b]	B	399(0.89)	389(0.58)	377(1.00)	366(0.62)	358(0.68)	...	...	7	19
2	Sinensiaxanthin[b]	B	425(0.89)	415(0.54)	400(1.00)	388(0.58)	379(0.68)	299(0.12)	...	10	18
3	Trollixanthin (?)	E	470(0.86)	458(0.72)	441(1.00)	...	419(0.74)	329(0.20)	315(0.15)	15	31
4	*cis*-Trollixanthin (?)	E	466(0.81)	457(0.69)	439(1.00)	...	416(0.69)	329(0.18)	317(0.14)	15	14
5	Valenciachrome[b]	B	375	363	354	344	337	...	...	15	2
6	Trollichrome (?)	E	450(0.91)	438(0.63)	422(1.00)	407(0.66)	399(0.72)	314(0.24)	300(0.21)	25	15

[a] B = benzene; E = ethanol.
[b] Proposed name.

mono-*cis* mutatoxanthin. The properties of the products formed on treatment with hydrochloric acid confirmed the tentative identification of fraction IIIB1 as antheraxanthin. While the latter has previously been found in nature in the anthers of *Lilium tigrinum* (*10*), and in the fruit of *Euonymus europaeus* (*9*), it is apparently one of the major pigments of Valencia orange juice.

The absorption maxima in benzene of fraction IIIB1 are close to those reported by Strain (*17*) for isolutein (486, 457 mμ) and position of IIIB1 on a magnesia column (between zeaxanthin and lutein) was also similar to that of isolutein. This suggests the possibility that isolutein and antheraxanthin are identical for the reported melting points (197° to 198° C., admittedly somewhat low, and 211° C.) are in the same vicinity. However, the carbon values (but not the hydrogen values) given by Strain for isolutein are nearer those for $C_{40}H_{56}O_4$ than for $C_{40}H_{56}O_3$. The spectral absorption maxima reported by Strain for isolutein are somewhat lower in wave length than those reported for antheraxanthin (*10*); this may be the result of the different instruments used. Smith pointed out (*15*) that values for the maxima obtained with a spectrophotometer may be several millimicrons lower than those obtained with a visual spectroscope where a copper ammonium filter is used. Chatterjee and Zechmeister (*1*) gave data on the maxima of several carotenoids using both a spectrophotometer and a visual spectroscope provided with a filter, and, for the highest wave length maxima, values obtained with the spectroscope were 5 to 7 mμ higher.

Fraction IIIB2, *cis*-Antheraxanthin The spectral absorption curve of this fraction indicated that it might be lutein epoxide (*5*). The maxima were several millimicrons lower than those of IIIB1, and the cis-peak was very low; the curve had relatively lower minima than those of IIIB1, and resembled those of lutein and IIIC1 (violaxanthin), except for the double cis-peak in the latter. A mixed chromatogram of fraction IIIB2 and IIIC1 resulted in ready separation into 2 bands, the lower of which proved to be IIIC1. Lutein epoxide on treatment with hydrochloric acid was transformed into two isomeric furanoxides, flavoxanthin and chrysanthemaxanthin (*5*). When fraction IIIB2 was treated with hydrochloric acid, and the resulting product chromatographed, five bands were obtained, the spectral absorption curves of which were very similar to those obtained from IIIB1 (Table II). Fractions IIIB1 and B2 evidently were stereoisomers; IIIB2 was probably a mono-*cis*-antheraxanthin and certainly not lutein epoxide (*5*). *cis*-Antheraxanthin has been found in nature in *Lilium candidum* (*19*); the spectral absorption curve had relatively lower minima than those of antheraxanthin, and resembled that of IIIB2 closely, except for the somewhat higher wave length maxima (487, 457 mμ).

Fractions IIIB3, IIIB4, and IIIB5, Mutatoxanthins Fractions IIIB3 and IIIB5 had very similar spectral absorption curves and are probably stereoisomers with the same configuration of the conjugated system. Fraction IIIB4 had slightly lower wave length absorption maxima and a higher cis-peak, and was probably a mono-cis compound closely related to IIIB3 and IIIB5. IIIB3 and IIIB5 were very probably stereoisomers, one of which was mutatoxanthin, and are referred to as mutatoxanthins *a* and *b*. Mutatoxanthin has apparently not been reported in nature, but it might accompany antheraxanthin in small amounts when the latter occurs in an acid fruit.

Fraction IIIB evidently consisted of stereoisomers of the epoxide and isomeric furanoxide of zeaxanthin (antheraxanthin and mutatoxanthin, respectively). These compounds contain two hydroxyl groups and one cyclic ether group.

Identification of Components of Fraction IIIC (Diether Diols)

Fraction IIIC6, Auroxanthin The spectral absorption curve of this fraction had maxima which corresponded to those of auroxanthin (zeaxanthin difuranoxide) (*4*) and were not at all close to those of any other known xanthophyll. Fractions with almost identical absorption spectra were obtained from the products of hydrochloric acid treatment of fractions IIIC1 to 5. Fraction IIIC6 was the most difficult to elute from a chromatographic column of any neutral carotenoid fraction obtained from orange juice.

Fraction IIIC1, Violaxanthin The spectral absorption curve showed that this fraction might be violaxanthin (*5*); on treatment with hydrochloric acid this compound formed auroxanthin, mutatoxanthin, and

a little zeaxanthin (*11*). Fraction IIIC1 on treatment with hydrochloric acid in methanol, and chromatography of the resulting product, yielded seven components. The first two were apparently mixtures with a number of low maxima. Components 3 and 4 had maxima which corresponded to those of mutatoxanthin, components 6 and 7 were two separate bands with practically identical absorption curves which matched those of auroxanthin (*5*, *11*). Component 5 was similar to 6 and 7 except the maxima were several millimicrons lower and the cis-peak was much higher; it appeared to be a mono-*cis*-auroxanthin. The absorption spectra of the products formed on treatment of fraction IIIC1 with hydrochloric acid confirm the identification as violaxanthin.

Fraction IIIC2, *cis*-Violaxanthin The absorption curve of this fraction was similar to that of IIIC1 except the maxima were shifted a few millimicrons toward shorter wave lengths and the curve had relatively lower minima and cis-peaks. On treatment with hydrochloric acid in methanol, and chromatography of the product, a series of fractions was obtained which had absorption spectra very similar to those of the fractions obtained in the same way from IIIC1. Fractions IIIC1 and IIIC2 evidently were stereoisomers. From the position of the absorption maxima, IIIC2 was a mono-cis isomer. Fraction IIIC2 may be the same as the violeoxanthinlike fraction of Moster *et al.* (*13*), and the violeoxanthin of Strain *et al.* (*18*). Products formed by action of acid on these fractions have apparently not been reported.

Fractions IIIC3, IIIC4, and IIIC5, Luteoxanthins The first two were present in rather small amounts and had practically identical absorption maxima. The third was present in somewhat larger quantity and had maxima a few millimicrons lower (allowing for the difference in solvents). The absorption maxima of IIIC3 and IIIC4 are in good agreement with those reported for flavoxanthin (lutein furanoxide) (*5*) and also for flavoxanthins *b* and *c* obtained from leaves by Strain (*17*). Like the latter IIIC3 and IIIC4 on chromatography on a magnesia column occurred well above violaxanthin. The spectral absorption curves of IIIC3, IIIC4, and IIIC5 resembled those of IIIC1, IIIC2, and IIIC6 in shape, including the two cis-peaks. Fraction IIIC3, on treatment with hydrochloric acid in methanol, yielded a main product, a substance with absorption spectrum very similar to that of auroxanthin, whereas if it had been flavoxanthin it would have been recovered unchanged, accompanied perhaps by some lutein. Fraction IIIC5 was also treated with hydrochloric acid in methanol, and on chromatography yielded products resembling those obtained from fractions IIIC1 and IIIC2.

Table II. Products Obtained on Hydrochloric Acid Treatment of Orange Juice Carotenoids

Fraction No.	Probable Identity of Reaction Products[a]	Wave Length, mμ, of Spectral Absorption Maxima (in Benzene)
	IIIB1 (Antheraxanthin)	
2	Zeaxanthin	488, 461, 345
3	Poly-*cis*-mutatoxanthin[b]	453, 427
4	Mutatoxanthin isomer	463, 436, 322
5	Mutatoxanthin isomer	464, 437, 320
	IIIB2 (*cis*-Antheraxanthin)	
2	Zeaxanthin	487, 461, 344
3	Poly-*cis* mutatoxanthin[b]	453, 427
4	Mutatoxanthin isomer	463, 436, 320
5	Mutatoxanthin isomer	464, 436, 322
	IIIC1 (Violaxanthin)	
3	Mutatoxanthin isomer	464, 435, 410
4	Mutatoxanthin isomer	463, 437, 412
5	*cis*-Auroxanthin	432, 406, 385, 303
6	Auroxanthin isomer	436, 409, 387, 301
7	Auroxanthin isomer	436, 409, 387, 302
	IIIC2 (*cis*-Violaxanthin)	
3	Mutatoxanthin isomer	463, 435, 410
4	Mutatoxanthin isomer	463, 436, 411
5	*cis*-Auroxanthin	434, 408, 386, 302
6	Auroxanthin isomer	436, 411, 388, 301
7	Auroxanthin isomer	436, 411, 387, 301
	IIIC3 (Luteoxanthin a)[c]	
Main	Auroxanthin	436, 410, 388, 301
	IIIC5 (*cis*-Luteoxanthin)[c]	
3	Mutatoxanthin	462, 435, 411
4	Auroxanthin isomer	435, 409, 387, 301, 290
5	Auroxanthin isomer	436, 410, 388, 301, 290
	IIID1 (Valenciaxanthin)[c]	
3	Valenchiachrome[c]	373, 354, 337
	IIID2 (Sinensiaxanthin)[c]	
2	Sinensiachrome[c]	404, 380, 360
	IIID3 (Trollixanthin)[b]	
3	Trollein[c]	485, 456, 432, 337
5	Trollichrome[b]	459, 430, 406, 318, 306

[a] Based on behavior on chromatography and on spectral absorption curves.
[b] Tentative identification.
[c] Proposed names.

Fractions IIIC1 to IIIC5 on hydrochloric acid treatment all yielded the same or very similar products according to the behavior on chromatography and the spectral absorption curves, and therefore must be closely related substances. Fractions IIIC3, IIIC4, and IIIC5 are intermediate between violaxanthin and auroxanthin. An experiment was carried out to see whether violaxanthin could be converted to substances resembling IIIC3, IIIC4, and IIIC5 by organic acids such as citric acid which is found in oranges.

A solution of violaxanthin (mainly fraction IIIC2 plus a small amount of IIIC1) was evaporated in vacuo and the residue dissolved in 5 ml. of a solution of 1% citric acid (hydrated) in methanol. After 2 minutes the solution was neutralized with 1 ml. of 20% potassium hydroxide in methanol, and the product chromatographed. Five fractions were obtained as shown in Table III. The first had absorption maxima similar to those of fraction IIIC2, the next three were similar to IIIC3, IIIC4 and IIIC5, and the last to auroxanthin. The approximate percentages of these—24, 74, and 2, respectively—indicated that under these conditions a major part of the violaxanthin had been converted to intermediate substances, but only a small part to auroxanthin, whereas with hydrochloric acid, auroxanthin is the principal product with little or none of the intermediates. This experiment confirms fractions IIIC3, IIIC4, and IIIC5 as intermediates between violaxanthin (zeaxanthin diepoxide) and auroxanthin (zeaxanthin difuranoxide). This also confirms the claim of Strain (*16*) that violaxanthin (from either pansies or leaves) on treatment with acid is converted first to flavoxanthins and then to auroxanthin. Fractions IIIC3 and IIIC4 are probably identical with flavoxanthins *b* and *c* of Strain (*17*), which he considered to be different from the Ranuculus flavoxanthin of Kuhn and Brockmann (*12*) and which Karrer and Jucker (*5*) claimed to be lutein furanoxide.

Fractions IIIC3, IIIC4, and IIIC5 (and also Strain's flavoxanthins *b* and *c*) therefore should be stereoisomeric forms

of zeaxanthin epoxide-furanoxide. This compound has apparently not been described but the corresponding dioxide (luteochrome) derived from β-carotene has been prepared (*6*), and had identical absorption maxima as those of α-carotene furanoxide (flavochrome) (*7*), which is the corresponding compound related to flavoxanthin. The term flavoxanthin has been applied to two substances (or groups of related substances) having similar absorption spectra, but different structures and molecular formulas. Because the substances, intermediate between violaxanthin and auroxanthin, are apparently analogous to luteochrome (β-carotene epoxide–furanoxide), it is proposed that these substances be referred to as "luteoxanthins." In the present work two fractions with practically identical absorption spectra were found, apparently having the same configuration of the conjugated double bond system but differing in some other respects. These are referred to as luteoxanthin *a* and luteoxanthin *b*.

Some references in the literature to flavoxanthin may refer to zeaxanthin epoxide–furanoxide and not to lutein furanoxide. This would probably be true in cases where violaxanthin was present, and when auroxanthin was also found. Auroxanthin is probably formed in vivo by the action of plant acids on violaxanthin, or may be formed during the working up of the material; however, in either case, the intermediate luteoxanthins would also be formed.

Fraction IIIC thus apparently consisted of stereoisomers of the diepoxide, epoxide-furanoxide, and difuranoxide of zeaxanthin (violaxanthin, luteoxanthins and auroxanthin, respectively).

Identification of Components of Fraction IIID (Monoether Polyols)

The wide gap between this fraction and fraction IIIC on countercurrent distribution suggests that the components of IIID must contain either a greater number of hydroxyl groups, probably three or/and four, or a considerably smaller number of carbon atoms, such as 25; β-citraurin, a 30-carbon compound, was found in orange peel by Zechmeister and Tuzson (*22*).

Fraction IIID1, Valenciaxanthin The positions of the maxima of the spectral absorption curve of this fraction indicated that this substance had at least one conjugated bond less than auroxanthin, which has seven in the central chain. Fraction IIID1 gave a fairly light blue color in the hydrochloric acid–ether test. On treatment with hydrochloric acid in methanol and chromatography of the product, only one fraction obtained was at all pure. This one had an absorption spectrum in benzene of maximum 373(0.93); minimum 364(0.59); maximum 354(1.00); minimum 344(0.59); maximum 337-(0.72); the maxima were in good agreement with those of fraction IIID5 and this fraction also had a bright greenish fluorescence in ultraviolet light. The drop in wave length of the absorption maxima caused by the hydrochloric acid treatment corresponded to that of the change of an epoxide to the corresponding furanoxide. The absorption maxima of the furanoxide were in good agreement with those of phytofluene, which are 367, 348, and 331 mμ in hexane (*21*). As in other epoxide–furanoxide pairs encountered in the present work, the hydrochloric acid conversion product required a considerably stronger eluent than the original compound (15 and 5% ethanol in petroleum ether, respectively). This difference is much more pronounced than that between an epoxide and the corresponding desoxy compound. Fraction IIID1 had absorption maxima which matched those reported for a fraction (390, 369, and 350 mμ in petroleum ether containing 6% acetone) obtained from Valencia orange juice by Natarajan and Mackinney (*14*). Fraction IIID1 apparently contained a system of six conjugated double bonds with an adjacent epoxide group and it is possible that this may be a carotenoid with fewer than 40 carbon atoms, perhaps 25. Because this is a previously undescribed substance, the name valenciaxanthin is proposed.

Fraction IIID5, Valenciachrome During the elution a strong greenish fluorescent zone in ultraviolet light was observed. When this fraction was eluted from the column, it contained colored material. The spectral absorption curve showed the presence of pronounced peaks at 375, 354, and 337 mμ in benzene. Because the fraction was not very pure, the relative absorbances of these maxima and minima in the ultraviolet are not given in Table I. Fraction IIID5 was identical with, or similar to, the hydrochloric acid conversion product of fraction IIID1 and the spectral absorption curve indicated that the latter was a much purer fraction. The absorption maxima of fraction IIID5 correspond closely to those of phytofluene so these two substances probably have the same chromophoric system of five conjugated double bonds. Fraction IIID5, however, must also possess several oxygen atoms, part of which must be hydroxyl groups, in order for it to occur in fraction IIID on countercurrent distribution. This substance apparently is the furanoxide corresponding to IIID1, and the name valenciachrome is proposed.

Fraction IIID2, Sinensiaxanthin This fraction had adsorption maxima in benzene which were 11, 9, and 8 mμ lower, respectively, than those obtained in benzene for auroxanthin. Fraction IIID2 gave a strong blue color in the hydrochloric acid–ether test, indicating the presence of two cyclic ether groups. On treatment with hydrochloric acid in methanol and chromatography of the product, the main fraction obtained had absorption maxima in benzene of 404, 380, and 360 mμ. This drop in the wave length of the absorption maxima corresponds to that of the conversion of an epoxide to a furanoxide group. This fact, the much more ready elution from a chromatographic column, and the occurrence in a widely separated fraction on countercurrent distribution, show the fraction IIID2 is not a stereoisomer of auroxanthin, but an unknown substance with a similar number of conjugated double bonds, and containing one epoxide group. The corresponding furanoxide (IIID2-2) was not found in fraction IIID, possibly because of its low absorptivity at visible wave lengths. This was the only instance in which an epoxide was isolated and the corresponding furanoxide was not found. Fraction IIID2 may be a carotenoid with less than 40-carbon atoms. The name sinensiaxanthin is proposed for this substance and sinensiachrome for the hydrochloric acid conversion product.

Fraction IIID3, Trollixanthinlike The absorption maxima of IIID3 were close to those of violaxanthin or lutein epoxide. This fraction gave a fairly strong blue color in the ether–hydrochloric acid test. On treatment with hydrochloric acid in methanol and chromatography of the product, two fairly pure fractions were obtained. The first had absorption maxima fairly close to those of lutein, but was eluted from the column by 15%

Table III. Products Obtained on Citric Acid Treatment of Violaxanthin

Fraction No.	Probable Identity[a]	Wave Length, mμ, of Spectral Absorption Maxima (in Benzene)	Approx. %
1	*cis*-Violaxanthin	477, 446, 422, 335, 321	24
2	Luteoxanthin[b]	460, 431, 406, 319, 302	6
3	*cis*-Luteoxanthin[b] isomer	456, 428, 404, 318, 304	20
4	*cis*-Luteoxanthin[b] isomer	455, 427, 404, 317, 304	48
5	Auroxanthin	434, 409, 386	2

[a] Based on behavior on chromatography and on spectral absorption curves.
[b] Proposed name.

ethanol in petroleum ether instead of 3.5% as for lutein. This fraction gave no color in the hydrochloric acid–ether test, and appeared to be a desoxy derivative still possessing at least three hydroxyl groups. The name trollein is proposed for this substance. The other fraction had absorption maxima quite similar to those of fraction IIID6, or about 20 mμ lower than those of IIID3, and apparently was the corresponding furanoxide. Fraction IIID3 was tentatively identified as trollixanthin, which was shown by Karrer and Krause-Voith (*8*) to be a trihydroxy monoepoxide, with spectral absorption properties resembling those of lutein epoxide.

Fraction IIID4, *cis*-Trollixanthinlike This fraction had spectral absorption maxima a few millimicrons lower than those of fraction IIID3, and was apparently a mono-cis isomer.

Fraction IIID6 Trollichromelike This fraction had absorption maxima very close to those of one of the hydrochloric acid conversion products of fraction IIID3. It was the most resistant to elution of any neutral fraction obtained from orange juice except auroxanthin. The properties of this compound are similar to those reported for trollichrome (*8*), which is apparently a trihydroxyfuranoxide with a similar chromophoric system to that of flavoxanthin.

Fraction IIID consisted primarily of an epoxide, tentatively identified as trollixanthin, together with a probably related mono-cis compound and also the corresponding furanoxide. Also present were two epoxides (valenciaxanthin and sinensiaxanthin) with very low wave length absorption maxima, together with the corresponding furanoxide (valenciachrome) of one of them.

Discussion

Little has been published previously on the polyoxygen carotenoids of oranges. Zechmeister and Tuzson (*22*) reported the presence of violaxanthin and probably flavoxanthin in Sicilian oranges. Natarajan and Mackinney (*14*) on the basis of absorption spectra and color tests tentatively identified two components of Valencia orange juice as lutein epoxide and flavoxanthin. The evidence cited by Natarajan and Mackinney favors equally the interpretation that *cis*-violaxanthin and a *cis*-luteoxanthin (fractions IIIC2 and IIIC5 in the present investigation) were actually the components found by these authors. Further experimental data obtained in the present investigation supports the latter interpretation.

The study of the 17 fractions obtained by chromatography from countercurrent distribution fractions IIIB, IIIC, and IIID, has indicated that seven of these were probably stereoisomers of other fractions. Of the ten remaining substances, there are three pairs of compounds apparently consisting of an epoxide and the corresponding furanoxide; also, a set of three compounds apparently consisting of a diepoxide, an epoxide-furanoxide, and a difuranoxide. The remaining compound was evidently an epoxide, for which the corresponding furanoxide was not found. Epoxides and the corresponding furanoxides generally occur together in plants, the latter probably being formed in vivo by the action of plant acids, or may be, at least in part, artifacts formed in working up the material. In the present work five parent epoxides were found: antheraxanthin, and violaxanthin, the mono- and diepoxides of zeaxanthin; a polyhydroxy compound tentatively identified as trollixanthin; and two substances (for which the names valenciaxanthin and sinensiaxanthin are proposed) with very low wave-length absorption maxima, apparently polyhydroxy compounds. It is possible that of the 17 components, only the five parent epoxides occur as such in orange, the others being formed by isomerization during or subsequent to the extraction of the juice. It is also possible that isomerization occurs in the orange before extraction.

In the work of this laboratory on the separation of the carotenoids of Valencia orange juice by chromatography without preliminary separation into fractions by countercurrent distribution, several components found in the present work were not detected. This is not surprising in view of the complexity of the xanthophyll fraction, which in the present work was separated into five fractions by countercurrent distribution before chromatography.

In previous work on the chromatography on magnesia of the hydrocarbon monol, and diol fractions (*2*), there were no instances in which a given carotenoid was isolated in more than one stereoisomeric form. In the present work on the cyclic ether di- and polyhydroxy carotenoids, most of the carotenoids isolated were obtained in two, or sometimes even three forms. In several cases two bands were obtained with identical or nearly identical absorption spectra; this occurred only with fractions believed to be furanoxides. When an epoxide is changed to a furanoxide by the action of acid, two stereoisomers may result, probably involving the hydroxyl group and the furan ring. When an epoxide is synthesized in nature apparently only one stereoisomer is formed in which the central chain is all-trans. Both epoxides and furanoxides occurred as forms in which the absorption maxima of one form were several millimicrons lower than those of the other, apparently due to the formation of a cis-bond in the central chain. These apparently formed more readily, or were more readily separable by chromatography, than mono-cis compounds of the carotenoids not containing cyclic ether groups. In some cases these stereoisomers with lower wave-length absorption maxima had lower cis-peaks than the higher wave-length absorption maxima isomers.

Approximate values for the percentage of the total carotenoids were calculated for some of the components, disregarding stereoisomers. The following percentages were obtained: antheraxanthin 20, violaxanthin 16, zeaxanthin 15, cryptoxanthin 9, lutein 7, mutatoxanthins 6, and luteoxanthins 5.

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