The Carotenoids of Red Bell Peppers

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The carotenoids in red bell peppers have been examined. Capsanthin accounted for about 35% of the total, with β-carotene and violaxanthin about 10% each, cryptoxanthin and capsorubin about 6% each, and cryptocapsin about 4%. Numerous other carotenoids were present in amounts of 2% or less, including at least eight apparently undescribed previously and two others not found previously in nature. All 10 of these pigments appear to contain cyclopentane rings as does capsanthin, capsorubin, and apparently cryptocapsin. In addition to capsanthin and capsorubin, five other constituents were found to contain keto groups. Capsanthin is distinctly different from the deep red pigment (reticulataxanthin) in tangerine and orange peels.

A PIGMENT that resembled capsanthin was found in the diol carotenoid fraction of the peels of Valencia (*5*) and navel (*8*) oranges, and in much greater quantity in tangerines (*6*). Capsanthin is the major pigment of the paprika, a variety of *Capsicum annuum* (*15*). Paprikas and other red varieties of *C. annuum* are very rich sources of carotenoids, particularly capsanthin and capsorubin. These two substances, which occur almost exclusively in *Capsicum* fruits, contain one and two keto groups, respectively. Zechmeister and von Cholnoky (*17*) published a series of papers on the paprika carotenoids; in addition to capsanthin and capsorubin, β-carotene, cryptoxanthin, and zeaxanthin were found. Cholnoky and coworkers (*2*) reported a number of additional paprika constituents, including cryptocapsin, lutein epoxide, antheraxanthin, violaxanthin, and mutatoxanthin. The structural formulas of capsanthin and capsorubin have been the subject of considerable controversy. In 1960, Entschel and Karrer (*9*), and Barber and coworkers (*1*) showed that capsanthin and capsorubin contain one and two cyclopentane rings, respectively, adjacent to the keto groups (Figure 1) which are a part of the conjugated double bond system. It was not known previously that the cyclopentane ring occurred in carotenoids.

An investigation has now been made of the carotenoids of red bell peppers, a variety of *C. annuum*. The major pigment, capsanthin, was found to be distinctly different from the tangerine pigment; eight apparently new carotenoids were found.

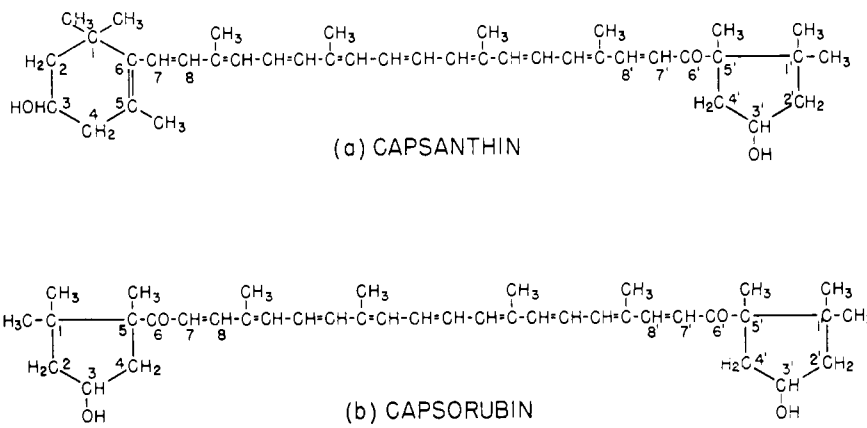

Figure 1. Partial structural formulae of (a) Capsanthin and (b) Capsorubin

Experimental

Two lots of red bell peppers (1200 grams each) were obtained in November; one was grown locally and the other obtained at a local market. Batches of 200 grams were each blended with 300 ml. of water and 3 grams of magnesium carbonate, then mixed with 500 ml. of methanol. Celite 503 was added (10% by weight of the fruit), the mixture filtered, and the filter cake processed as previously described, including saponification by potassium hydroxide in a one-phase system of methanol-ether (*7*).

The following solvent systems were used in countercurrent distribution runs in a Craig apparatus: I, hexane (Skellysolve B) and 99% methanol, 1.8 to 1 by volume; II, benzene, hexane, and 87% methanol, 1 to 1 to 1.15 by volume (*3*); III, hexane, acetone, methanol, and water, 1.25 to 1 to 0.1 to 0.65 by volume (*4*); and IVA, hexane and 75% methanol, 1 to 1 by volume. The last system is similar to the system hexane and 73.5% methanol previously described (*4*).

Fractions obtained by countercurrent distribution were further fractionated by chromatography on columns of magnesia (Sea Sorb 43), 14 × ca. 90 mm., without a diluent. A graded series of eluants (*7*) was used. Spectral data for the resulting bands were obtained with a Beckman DK-2 recording spectrophotometer.

Hydrochloric Acid–Ethanol Test for 5,6-Epoxides. An aliquot of the carotenoid solution having an absorbance equivalent of about 0.9 in 5 ml. of solution is evaporated in vacuo in a rotary evaporator, and the residue dissolved in 5 ml. of absolute ethanol. The spectral absorption is measured from 550 to 300 mμ, then one drop of concentrated hydrochloric acid is added to the solution in the cuvette, and the spectral absorp-

Table I. Fractionation of Red Bell Pepper Carotenoids by Countercurrent Distribution with Solvent System I

Fraction	% of Total Carotenoids[a]	N_{100} Value[b]
I, hydrocarbons	11.3	91
IIA, monols	3.3	56
IIB, monoketo-monols	1.0	38
III, diols and polyols	84.3	5

[a] As measured in an Evelyn photoelectric colorimeter with filter 440.
[b] Tube number of maximum per 100 transfers.

tion again measured. A decrease in the wave length of the spectral absorption maxima of about 20 mμ indicates the presence of one 5,6-epoxide group, whereas with two groups the decrease is about 40 to 45 mμ (*12*). Carotenoids not containing 5,6-epoxide groups usually show a decrease of 1 to 2 mμ.

Test for Allylic Hydroxyl Groups. A carotenoid solution is evaporated, and the residue dissolved in a mixture of 9 ml. of methanol and 1 ml. of concentrated hydrochloric acid. The solution is allowed to stand for 2 to 10 minutes, then an excess of potassium hydroxide in methanol is added. A countercurrent distribution run is carried out on the recovered carotenoid in an appropriate solvent system. A considerable increase in N_{100} value (tube number of maximum per 100 transfers) indicates the presence of an allylic hydroxyl group. Petracek and Zechmeister (*14*) showed that some allylic hydroxyl groups in carotenoids will form methyl ethers on treatment with acidic methanol.

Sodium Borohydride Reduction of Ketonic Carotenoids. Reductions were carried out with a number of carotenoids which apparently contained keto groups, by a method similar to that of Krinsky and Goldsmith (*13*). To a solution of the carotenoid in 95% ethanol in a nearly filled screw-cap bottle (usually 0.5 ounce) is added several lumps of sodium borohydride. The solution is allowed to stand in the refrigerator overnight with the screw cap slightly loosened, then it is added to about 100 ml. of ether, and the ether solution washed several times with water. 5,6-Epoxides are not reduced under these conditions. Warren and Weedon (*16*) reduced capsorubin and capsanthin with potassium borohydride in boiling methanol.

Results and Discussion

The total carotenoid contents of the two lots of peppers were 284 and 127 mg. per kg. (as β-carotene).

Countercurrent Distributions. The carotenoids from the first lot of fruit in a 100-transfer run with system I (Table I) showed a predominance of diols and polyols (fraction III). Much smaller amounts of hydrocarbons (I) and monols (IIA) were present, and there was a minor fraction (IIB) with N_{100} value of 38. Fractions I, IIA, and IIB were examined chromatographically; the principal components were found to be β-carotene, cryptoxanthin, and cryptocapsin (a monoketomonol) (*2*), respectively. Fraction III was not chromatographed, but was further fractionated by means of countercurrent distribution runs with solvent systems II and IVA.

Earlier results with system II showed the N_{100} values of the diol, monoepoxide diol, diepoxide diol, and polyol fractions to be about 69, 55, 40, and 9, respectively. In the present study, four maxima with N_{100} values of 67, 45, 23, and 10 were observed. By means of chromatographic examination, the major one at 45 was found to be due to capsanthin, the lesser one at 23 to capsorubin. The capsanthin fraction obscured the diepoxide diol (IIIC) and the monoepoxide diol (IIIB) fractions, the positions of which are indicated on the curve in Figure 2. The capsorubin fraction was found in the usual gap between the polyol and diepoxide diol fractions. The introduction of keto groups in the diol molecule resulted in a considerably greater change in N_{100} values than for epoxide groups: from 69 to 45 and 23 for mono- and diketones *vs.* 55 and 40 for mono- and diepoxides.

With system IVA the N_{100} values of the diol, monoepoxide diol, diepoxide diol, and polyol fractions from other fruits had been found to be about 79, 47, 17, and 0,

Table II. Carotenoids Obtained from Red Bell Peppers

(Values in parentheses are for shoulders or humps on spectral absorption curves)

	Carotenoid	Spectral Absorption Maxima,[a] Mμ	Approximate %	N_{100} Value[b]
I-1	Phytoene	(298), 286, (276)	1.7	
I-2	Phytofluene	366, 348, 332	1.1	
I-3	α-Carotene	486, 456, 431, 339[c]	0.2	
I-4	β-Carotene	447, 449, (424), 338	11.6	[I(91)] [II(97-8)]
I-5	ζ-Carotene	423, 397, 377	1.5	
I-6	Mutatochrome-like	451, 428, 402	0.3	
IIA-1	Hydroxy-α-carotene	473, 444, 420, 332	1.0	
IIA-2	Cryptoxanthin	476, 447, (422), 338	6.7	[I(56)] [II(96)]
IIA-3	Hydroxy-α-carotene-like	473, 445, (423), 333	0.4	
IIB-1	Cryptoflavin-like	451, 426, (402)	0.5	
IIB-2	Cryptocapsin	(497), 470, (445), 353	4.3	I (38)
IIIA-1	Capsolutein	486, 455, (430), 339	2.3	II(66); HCl, I(10)
IIIA-2	Zeaxanthin	493, 463, (437), 346	2.3	[I(11)]; II(69); HCl, I(13)
IIIB-1	P-482, diol[d]	482, 451, 424, 336, 320	0.6	II(63); HCl, I(8)
IIIB-2	Capsolutein-5,6-epoxide[e]	483, 451, 425, 336, 322	1.5	II(52); HCl, I(7)
IIIB-3	Antheraxanthin	487, 456, (431), 338	1.6	[II(55)]
IIIB-4	Capsolutein-5,8-epoxide[e]	459, 430, 406, 318, 303	0.4	II(54)
IIIB-5	Mutatoxanthins	464, 437, (412), 320	1.6	[II(54)]
IIIC-1	Violaxanthin	484, 452, 425, 336, 321	9.9	[II(40)]
IIIC-2	Luteoxanthins	460, 431, 406, 319, 303	0.9	[II(40)]
IIIC-3	Capsanthin	(510), 483, 363	34.7	II(45), HCl, II(46)
IIID-1	P-441, tetrahydrocapsorubin[e]	441, 414, 392	0.7	II(23); III(81)
IIID-2	Hydroxycapsolutein[e]	486, 456, (430), 338	1.9	II(26); III (87); HCl, II (26)
IIID-3	Capsanthin 5,6-epoxide[e]	(509), 478, (455), 357, 344	0.9	II(34)
IIID-4	Capsochrome[e]	(483), 456, (431), 345	0.3	
IIID-5	Capsorubin	522, 487, (460)	6.4	II(23); III(78)
IV-1	Hydroxycapsolutein-5,6-epoxide[e]	482, 449, 421, 336, 321	1.0	II(16); III(75)
IV-2	Neoxanthin	478, 447, 421, 335, 321	0.7	III(61)
IV-3	Trolliflor-like	482, 449, 423	0.1	III(30)
IV-4	Carbonyl[f]	458	1.0	
IV-5	Hydroxycapsanthin-like	478, 358	0.5	II(10); III(60); HCl, II(10)

[a] I, IIA, IIB in hexane; others in benzene.
[b] See Table I; system number in Roman numerals, earlier results with carotenoids from other sources in brackets.
[c] In benzene.
[d] P-482 refers to a pigment with highest wave length spectral absorption maximum at 482 mμ.
[e] Tentative identification.
[f] Spectral absorption curve with one maximum.

Table III. Properties of Some Bell Pepper Carotenoids, Their $NaBH_4$ Reduction Products, and Hydrochloric Acid Conversion Products

Carotenoid	Spectral Absorption Maxima, Mμ (Benzene)	N_{100} Values[a]
Cryptocapsin	(497), 470, 353[b]	I(38)
Cryptocapsol	483, 454, (428), 338	I(22); II(85)
Dehydrocryptocapsol	496, 464,, 438, 369	I(56)
Capsanthin	483, 363	II(45)
Capsanthol	482, 452, (428), 337	II(22); III(89)
Dehydrocapsanthol	495, 464, 439, 368	I(39); II(93)
Capsanthin 5,6-epoxide[c]	478, 357	II(34)
Capsanthol 5,6-epoxide[c]	482, 451, 427, 336, 319	II(15); HCl, II(53)
P-441	441, 414, 392	II(23); III(81)
Reduced P-441	442, 414, 392	II(2), HCl, II(3)
Capsorubin	522, 487, (460)	II(23), III(78)
Capsorubol	481, 449, 424, 334, 320	II(4); III(59, 39)
Hydroxycapsolutein 5,6-epoxide[c]	482, 449, 421, 336, 321	II(16)
Hydroxycapsolutein-5,8-epoxide (*cis* ?)[c]	456, 428, 410	II(17)
Hydroxycapsanthin-like	478, 358	II(10), III(60); HCl, II(10)
Hydroxycapsanthol-like	480, 449, 424, 335, 320	III(52, 32)

[a] Solvent system identified by Roman numerals. [b] In hexane. [c] Tentative identification.

respectively. With the *Capsicum* carotenoids, the N_{100} values were at 75, 46, 23, and 1 (Figure 2). The major fraction, with N_{100} of 23, was due primarily to capsanthin, while capsorubin appeared in the polyol fraction. Again, the capsanthin fraction obscured the diepoxide diol fraction and, in part, the monoepoxide diol fraction. The slightly lower values of the diol fraction in both this and the run using system II were found to be significant, since these fractions contained relatively large amounts of constituents with somewhat lower N_{100} values than lutein and zeaxanthin. With both systems II and IVA, the presence of capsanthin and capsorubin considerably complicated the fractionation by countercurrent distribution.

Carotenoids from the second lot of fruit were used in a 200- transfer run with solvent system II. The contents of the various tubes were combined as follows: polyol (IV) 0 to 28; capsorubin (IIID) 29 to 56; diepoxide diol (IIIC) 61 to 88; monoepoxide diol (IIIB) 98 to 119; diol (IIIA) 129 to 156; and hydrocarbon-monol (I-II) 166 to 199. In this run, the tubes above No. 56 were combined as was done for other fruits where capsanthin and capsorubin were absent. Because there was considerable color in them, the intermediate tubes also were combined into four fractions.

The six main and four interband fractions from the 200-transfer run with system II were each chromatographed. Components obtained are listed in Table II. Some of the carotenoids, especially capsanthin, occurred in more than one fraction; these are listed under the fraction in which they occurred to the greatest extent.

Hydrocarbons (I). No unusual components were obtained from fraction I. β-Carotene was the second most abundant of all the carotenoids. A mutatochrome-like fraction was reported also by Cholnoky and coworkers (*2*), but they did not list phytoene, phytofluene, α- and ζ-carotenes

Monols (II). The most abundant component of fraction II was the previously reported cryptoxanthin. The other major constituent had a spectral absorption curve similar to that of capsanthin, with a maximum at 470 mμ (in hexane) and a prominent shoulder at 497 mμ. This was identified as crytocapsin, as reported by Cholnoky and coworkers (*2*).

On treatment of crytocapsin with sodium borohydride, the color of the solution became much less red. The spectral absorption maxima of the product cryptocapsol (Table III) were similar to those of a *cis*-lutein, indicating reduction of a carbonyl group conjugated with the central double bond system. The N_{100} values of cryptocapsol in systems I and II, respectively, were 22 and 85, as compared with 10 and 69 for lutein. The second hydroxyl group in cryptocapsol is apparently considerably less exposed than in lutein, probably being in the 6′ position (Figure 1) instead of 3 and 3′; it is also allylic.

Cryptocapsol was treated with hydrochloric acid in methanol for 5 minutes, and the product used in a countercurrent distribution run with system I. The main product had an N_{100} value of 56, in close agreement with that of cryptoxanthin, a 3-hydroxy-carotene. The spectral absorption maxima were at higher wave lengths (Table III), indicating that instead of forming a methyl ether as in lutein, a molecule of water had been split off with the addition of a double bond to the conjugated system and the formation of a dehydrocryptocapsol, the double bond system now apparently extending from carbon 6′ to carbon 4 (Figure 1*a*). If the remaining hydroxyl group were on carbon 3, it would be allylic and would be readily methylated under these conditions to form a substance with an N_{100} value of about 85. The fact that this was not formed indicates that the hydroxyl group in cryptocapsin is probably in the cyclopentane ring on the 3′ carbon, and that cryptocapsin is apparently 3-deoxycapsanthin; this has an unsubstituted β-ionone ring characteristic of a provitamin A. In dehydrocryptocapsol, the double bond system has been shifted over one carbon from that in capsanthin (Figure 1*a*) to what is called a retro system. This shift is confirmed by the presence of a minor peak at 369 mμ, whereas normal carotenoids usually have a *cis*-peak at a wave length 142 ± 2 mμ lower than that of the highest wave length maximum, or at ca. 352 mμ.

Several minor components also occurred in fraction II. Hydroxy-α-carotene-like bands occurred both below and above the cryptoxanthin band. The hydrochloric acid–ethanol test for 5,6-epoxides was negative for both of these substances, showing they were not a spectrally similar cryptoxanthin-5,6-epoxide.

Diols (IIIA). The first constituent eluted from the column in this fraction had a spectral absorption curve resembling that of violaxanthin; the hydrochloric acid–ethanol test for 5,6-epoxides was negative, however. This constituent was found in somewhat greater quantity in fraction IIIB and the interband fraction, and is therefore listed in Table II as IIIB-1 (P-482). However, since it is, apparently, a diol without epoxide or ketone groups, it is discussed here. The N_{100} value of P-482 in system II (63) was significantly lower than that of zeaxanthin (69). On treatment with hydrochloric acid in methanol, the product had an N_{100} value of 8 in system I, indicating the absence of allylic hydroxyl groups. This rules it out as a dihydroxy-ε-carotene, which would differ from zeaxanthin in having α-ionone instead of β-ionone rings. The lower N_{100} value indicates that the hydroxyl groups are in a slightly more exposed position than in zeaxanthin. A possibility is that P-482 is derived from capsorubin (Figure 1*b*) by replacing both carbonyl groups with CH_2 groups, and retaining both cyclopentane rings.

The second component (IIIA-1) of fraction IIIA appeared to be lutein. A similar substance was found in smaller amount in fraction IIIB. The N_{100} value of the lutein-like component was 66 as compared with 69 for zeaxanthin and lutein, a small but probably significant difference. The hydrochloric acid–ethanol test for 5,6-epoxides was negative. After treating IIIA-1 with hydrochloric acid in methanol and

carrying out a countercurrent distribution of the product with system I, the N_{100} value was 10, identical with that of lutein. But lutein has an allylic hydroxyl group and, after hydrochloric acid–methanol treatment, the N_{100} value was 38 (lutein monomethyl ether). There was not even a minor component at N_{100} ca. 38 in the hydrochloric acid–methanol treated fraction IIIA-1, showing that it was not lutein and did not contain even a significant impurity of lutein. When an aliquot of fraction IIIA was treated with hydrochloric acid in methanol and the product used in a countercurrent distribution run with system I, a component was found with N_{100} value of 39, which may have consisted, at least in part, of lutein methyl ether. The amount of this component was less than 2% that of the diol maximum (at N_{100} of 11). This indicates that not more than a trace of lutein was present in fraction IIIA. The name capsolutein is proposed for IIIA-1. Cholnoky and coworkers (*2*) did not report lutein or a lutein-like pigment from red paprikas though they did find lutein in green paprikas. The N_{100} value of IIIA-1 in system II is halfway between the values for P-482 and zeaxanthin. Capsolutein may be derived from capsanthin (Figure 1*a*) by replacing the carbonyl group with a CH_2 group.

Monoepoxide Diols (IIIB). The first four bands emerging from the column with fraction IIIB were P-482, a lutein 5,6-epoxide-like substance, capsolutein, and antheraxanthin. The second and fourth of these gave positive 5,6-epoxide tests. The N_{100} value of the second band in system II was 52 as compared with 55 for antheraxanthin. After treatment of band 2 with hydrochloric acid–methanol, the N_{100} value in system I was 7, indicating that this substance, like capsolutein but unlike lutein 5,6-epoxide, contained no allylic hydroxyl group, and that it is probably capsolutein 5,6-epoxide, with one cyclopentane ring. Because the 5′-carbon of capsanthin is tertiary, the double bond system cannot extend into the cyclopentane ring, and substances with spectral absorption properties like zeaxanthin cannot exist with the cyclopentane ring. This applies also to the mono- and diepoxides of zeaxanthin (antheraxanthin and violaxanthin). The 5,8-epoxides corresponding to capsolutein 5,6-epoxide and antheraxanthin were both present in this fraction.

Diepoxide Diols (IIIC). Fraction IIIC contained most of the capsanthin, which had an N_{100} value in system II of 45 as compared with 40 for violaxanthin and 67 for reticulataxanthin, the deep red pigment of tangerine peel. Violaxanthin and the corresponding 5,6,5′,8′-diepoxides were both present in this fraction; in the hydrochloric acid–ethanol test both were converted to auroxanthins the 5,8,5′,8′-diepoxides.

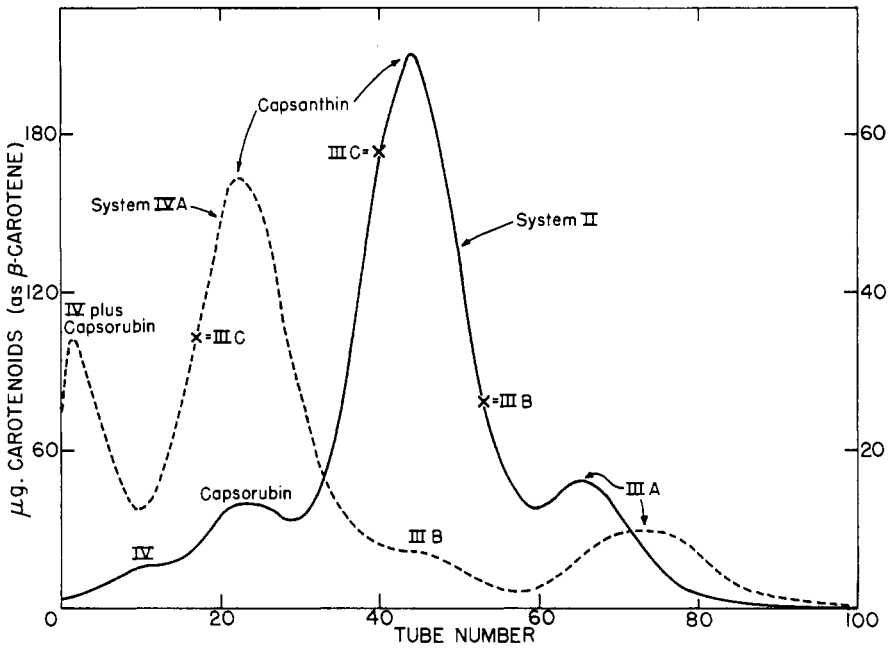

Figure 2. Countercurrent distribution of diol-polyol fraction of bell pepper carotenoids with solvent systems II and IVA

Capsanthin was reduced by sodium borohydride to capsanthol, previously prepared by Karrer and Hübner (*10*), which had spectral absorption values similar to those of a *cis*-lutein (Table III). There was a considerable decrease to 22 in N_{100} value in system II. After capsanthol was treated with a solution of hydrochloric acid in methanol for 10 minutes, the N_{100} of the main product, in system II, was 93, and in system I, 39 (lutein methyl ether was 38). The spectral absorption maxima of the main product in benzene were at higher wave lengths than those of capsanthol, showing that here, as with cryptocapsol, a dehydration had taken place. In the resulting product, the 3-hydroxyl group was now allylic and formed a methyl ether, so that the final product was apparently 3-methoxy-4,6′-dehydrocapsanthol.

Capsorubin Fraction (IIID). Fraction IIID was separated visually so as to include practically all of the deep red capsorubin. In carotenoid mixtures from other fruits, there is usually a minimum of color in this range. On chromatography of this fraction, several components besides capsorubin were found. The first one eluted from the column was P-441 (Table II), the spectral absorption maxima of which are not in agreement with those of any carotenoid previously isolated by the author, being about 4 to 5 mμ higher than those of auroxanthin. P-441 probably has the same number of conjugated double bonds (seven) as auroxanthin. The spectral absorption curve of P-441 in ethanol was similar in shape to that in benzene, with much fine structure, indicating that the conjugated double bond system does not include a carbonyl group. On treatment with hydrochloric acid in ethanol, the wave length of the spectral absorption maxima showed little change, indicating the absence of 5,6-epoxide groups. The N_{100} values of P-441 in both systems II and III were close to those of capsorubin; on treating P-441 with sodium borohydride, the spectral absorption maxima in benzene were practically unchanged (442, 414, and 392 mμ), but the N_{100} value in system II was changed from 23 to 2 (capsorubin changed from 23 to 4). After treatment of reduced P-441 with hydrochloric acid in methanol, the N_{100} value was 3. This indicates the presence in P-441 of two carbonyl groups not in conjugation with the central double bond system, since on reduction the hydroxyl groups formed were not allylic. It is suggested that P-441 is 7,8,7′,8′-tetrahydrocapsorubin.

The second band (IIID-2) had spectral absorption maxima similar to those of lutein. The N_{100} values in systems II and III were in fair agreement with those of capsanthol (Table III), but the behavior of IIID-2 on treatment with hydrochloric acid in methanol was quite different. The N_{100} value of IIID-2 was unchanged, whereas that of capsanthol in system II was changed from 22 to 93. Fraction IIID-2 therefore cannot have a hydroxyl group on carbon 6′ (Figure 1). The spectral absorption maxima showed no significant shift on addition of hydrochloric acid to a solution of IIID-2 in ethanol. The N_{100} value of IIID-2 of 87, in system III, was higher than those of trolleins *a* and *b* (*4*) (two polyols

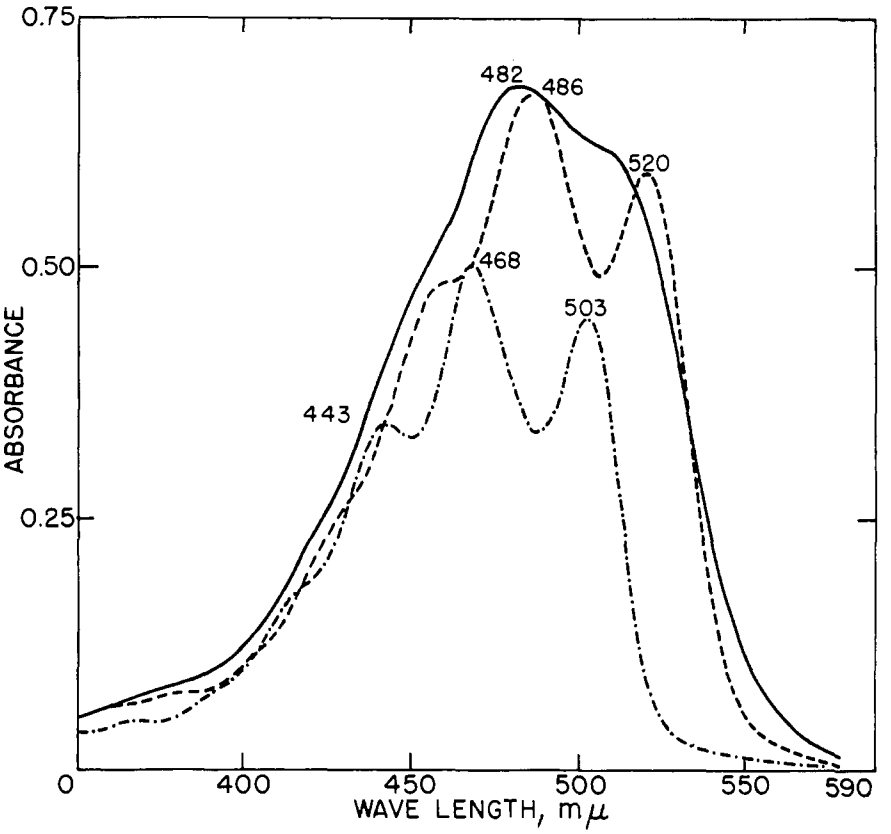

Figure 3. Spectral absorption curves for capsorubin

——— in ethanol
– – – – in benzene
–·–·–·– in hexane

obtained from oranges with lutein-like spectral absorption characteristics) which were 73 and 55, respectively. IIID-2 appears to be a derivative of capsolutein with a third hydroxyl group on a rather highly protected carbon. Hydroxyl groups on the methyl groups or on carbon 2 of the cyclohexane or cyclopentane rings are unknown in naturally occurring carotenoids. A hydroxyl on carbon 4 of the cyclohexane ring would be allylic, therefore a third hydroxyl group would probably be present on carbon 4′ of a cyclopentane ring.

IIID-3 resembled capsanthin except that the spectral absorption maxima, including that of the *cis*-peak, were several mμ shorter in wave length. The N_{100} value was 34 as compared with 45 for capsanthin and 23 for capsorubin. On adding hydrochloric acid to a methanol solution of IIID-3, a deep blue-green color formed, indicating the presence of an epoxide. In ethanol solution, the spectral absorption maxima changed from 477 and 358 to 449 and 336 mμ on addition of a drop of hydrochloric acid, thus indicating the conversion of a 5,6-epoxide to a 5,8-epoxide. After reduction of IIID-3 with sodium borohydride, the maxima were at 482, 451, and 427 mμ in benzene (similar to violaxanthin); the N_{100} in system II changed from 34 to 15. After treating the reduced product with hydrochloric acid in methanol, the N_{100} of the main product in system II shifted from 15 to 53 (corresponding to a diol monoepoxide), a loss of the 6′ hydroxyl group apparently occurring. Fraction IIID-3 is apparently capsanthin 5,6-epoxide, which has been made synthetically from capsanthin by Karrer and Jucker (*11*); IIID-4 is apparently the corresponding 5,8-epoxide capsochrome.

The principal component of fraction IIID was the diketone capsorubin. There appears to be no published spectral absorption curve for this substance, hence curves are given in Figure 3 for solutions in hexane, benzene, and ethanol. The shape of the curve in hexane is similar to that of α-carotene, but the maxima are at much longer wave lengths. In benzene, the curve is somewhat less sharp, with the third maximum reduced to a shoulder. In ethanol, only one maximum is present. Spectral absorption curves of a given noncarbonyl carotenoid in these three solvents are usually quite similar in shape.

Capsorubin was reduced by sodium borohydride to capsorubol (Table III) which has spectral absorption properties similar to those of violaxanthin. It had an N_{100} in system II of 4. In system III, two maxima were present, the one at N_{100} of 59 being somewhat greater than the one of 39; these two fractions had similar spectral absorption curves. Both of these components apparently still have four hydroxyl groups, since capsanthol (a triol) had an N_{100} in this system of 89. It would be anticipated that on reduction of capsorubin, three stereoisomers would be formed, *dd*, *ll*, and *meso*. Perhaps one fraction consisted of the *meso* isomer, the other of the other two. An attempt was made to separate the combined capsorubol fraction by chromatography on Sea Sorb; a very strong eluant (20% methanol in benzene) was required, but no separation was observed except that the lower part of the band consisted mainly of a *cis*-isomer.

After treatment of the reduced capsorubin with hydrochloric acid in methanol, the N_{100} value of the main fraction in system II changed from 4 to 74, a value higher than that of zeaxanthin (69), a diol. The spectral absorption maxima of the recovered product were at 476, 444, and 418 mμ in benzene, corresponding approximately to those of a *cis*-isomer of the starting material. A loss of two hydroxyl groups apparently had occurred, but without change in the conjugated double bond system.

Polyols (IV). The lowest band on the column (IV-1) had a spectral absorption curve similar to that of violaxanthin. This substance occurred also in fraction IIID in somewhat smaller amounts. The hydrochloric acid–ethanol test showed it to be a mono-5,6-epoxide. The N_{100} values were higher than those of neoxanthin. Fraction IV-1 appeared to be the 5,6-epoxide corresponding to IIID-2, having moderately lower N_{100} values in both systems II and III.

The second band (IV-2), also a 5,6-monoepoxide, was identified as neoxanthin by the N_{100} value in system III of 61; a minor impurity in this band with N_{100} of 30 appeared to be a tetraol, possibly trolliflor.

Fractions IV-4 and IV-5 had spectral absorption curves with a single maximum in the visible region, like that of capsanthin. The maxima of IV-5 were similar to those of IIID-3 (apparently capsanthin-5,6-epoxide), but the N_{100} in system II was 10. This substance appears to have the same conjugated double bond system as IIID-3. The hydrochloric acid–ethanol test for epoxides was negative. After treatment of IV-5 with hydrochloric acid in methanol, the N_{100} in system II was unchanged, indicating the absence of an allylic hydroxyl group. Reduction with sodium borohydride yielded a substance with maxima at 480, 449, and 424 mμ (violaxanthin-like). Countercurrent distribution with system III revealed two maxima, as in reduced capsorubin, with

N_{100} values of 52 and 32, the former being somewhat greater in quantity. These values are lower by 7 than those obtained with capsorubin, indicating the probable presence of four hydroxyl groups, with one in a less hindered position than the 6 or 6′ positions in reduced capsorubin. After treatment of the reduced product with hydrochloric acid in methanol, the N_{100} of the main product in system II was 73 (the product obtained from reduced capsorubin had a value of 74). Apparently, it is a diol. It appears that IV-5 is a 6′-keto-3,3′,4′-triol.

The apparently undescribed carotenoids IIIA-1, IIIB-1, IIIB-2, IIIB-4, IIID-1, IIID-2, IV-1, and IV-5, as well as the substances tentatively identified as capsanthin-5,6-epoxide and capsochrome, were all present as minor constituents (0.4 to 2.3% of the total carotenoids) which will render more difficult the complete elucidation of their structures.

Seven components were found to contain ketone groups, capsanthin, capsorubin, cryptocapsin, capsanthin-5, 6-epoxide-like, capsochrome-like, P-441 (tetrahydrocapsorubin ?), and polyol IV-5.

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SUGAR CANE PHOSPHOLIPIDES

The Isolation, Separation, and Identification of the Principal Phospholipides of Sugar Cane Juice

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This investigation of the phospholipides of sugar cane juice is part of a broad study on nonsugars in cane juice. Because of the emulsifying nature of phospholipides, it is believed that these compounds have a deleterious effect on the processing and clarification of cane juice. By utilizing a combination of solvent fractionation and silicic acid column chromatography, the principal phospholipides were isolated from lyophilized fresh sugar cane juice and separated. They corresponded chromatographically to phosphatidyl ethanolamine and lecithin on silicated glass paper.

Determination of the composition of the total lipide fraction of sugar cane juice has been the object of numerous investigations, primarily because of interest in sugar cane wax which has commercial value. Balch (*2*) has compiled a fairly complete bibliography of the subject up to 1953. The phospholipide fraction of the total lipides has no commercial value as a by-product, but is thought to be of importance to the sugar industry because of the deleterious effect that phospholipides would have upon the formation and settling of the precipitate during clarification of the juice. Honig (*8*) points out that, since lecithin and other phosphatides are well known emulsifying agents, even small quantities of these substances in sugar cane juice would affect clarification adversely. Despite the apparent importance of these compounds, a review of the literature reveals a paucity of information. Shorey (*13*) undertook a study of a wax-like solid that he obtained from sugar cane juice in 1897; he found that this substance contained both phosphorus and nitrogen, and had the physical properties of lecithins. In another early study of sugar cane lipides, Wijnberg (*14*) reported the presence of lecithin in sugar cane wax. More recently, Hatt, Strasser, and Troyahn (*7*), also investigating the wax fraction of sugar cane, reported that the presence of glycerol and phosphorus could be accounted for by a phosphatide fraction. Their evidence, however, was indirect as they did not isolate phospholipides as such, nor did they identify the nitrogen-containing radicals usually associated with phospholipides. Honig's (*8*) study suggests that the phospholipide in sugar cane juice is a lecithin, but he points out that definite proof of its presence is still lacking.

In view of the scarcity of information on this important constituent of sugar cane juice, this investigation was undertaken as part of a broad study on the composition of nonsugars in cane juice.

Methods

Preparation of Material for Analysis. Samples of fresh, raw sugar cane juice were obtained from grinding individual varieties of cane under commercial conditions in the experimental Audubon Sugar Factory at Louisiana State University during pilot plant clarification studies as described by Guilbeau, Black, and Martin (*4*). The fresh juice was frozen and dried on a large capacity lyophilizer as reported by Roberts (*12*). Mixed lots of the dried juice, in quantities varying from 500 to 1000 grams, were extracted in a 1-gallon capacity blender using an ethanol–ethyl ether (2 to 1, v./v.) solvent system; the dried juice and solvent were blended at medium speed for approximately 5 minutes and were then allowed to stand for 3 hours. This mixture was filtered under vacuum through a large, coarse-