Carotenoid Composition in the Fruits of Red Paprika (*Capsicum annuum* var. *lycopersiciforme rubrum*) during Ripening; Biosynthesis of Carotenoids in Red Paprika

József Deli,* Péter Molnár, Zoltán Matus, and Gyula Tóth

Department of Medical Chemistry, University of Pécs, Medical School, H-7624 Pécs, Szigeti út 12, Hungary

The changes in the carotenoid pigments of the *Capsicum annuum* var. *lycopersiciforme rubrum* during maturation have been investigated quantitatively by means of a HPLC technique. In all of the chromatograms, 40 peaks were detected; 34 carotenoids were identified. The total carotenoid content of the ripe fruits was about 1.3 g/100 g of dry weight, of which capsanthin constituted 37%, zeaxanthin was 8%, cucurbitaxanthin A was 7%, capsorubin constituted 3.2%, and β-carotene accounted for 9%. The remainder was composed of capsanthin 5,6-epoxide, capsanthin 3,6-epoxide, 5,6-diepikarpoxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, and several cis isomers and furanoid oxides. The possible biosynthetic routes for the formation of minor carotenoids containing 3,5,6-trihydroxy-β-, 3,6-epoxy-β-, and 6-hydroxy-γ-end groups are described.

Keywords: *Paprika; carotenoids; analysis; biosynthesis; Capsicum annuum*

INTRODUCTION

Paprika, *Capsicum annuum*, is one of the oldest, most important, and widely used carotenoid food colorants. The red varieties of *C. annuum* are very rich sources of carotenoids, particularly capsanthin and capsorubin containing one and two keto groups, respectively.

The composition of the carotenoid pigments produced by paprika has been investigated for a long time. A detailed pioneer work (*1*) on the qualitative and quantitative distribution of carotenoids in red paprika (*C. annuum* var. *lycopersiciforme rubrum*) revealed that the red carotenoids (capsanthin, capsorubin, and cryptocapsin) are formed from the appropriate 5,6-epoxycarotenoids (antheraxanthin, violaxanthin, and cryptoxanthin 5,6-epoxide). At the time, the carotenoid composition of yellow paprika (*C. annuum* var. *lycopersiciforme flavum*) was also investigated, which, although containing a large amount of 5,6-epoxy carotenoids, was lacking in the red carotenoids (*2*).

The HPLC method was introduced to the research of carotenoids in the 1970s . Since then, a large number of applications on HPLC methods have been published. By means of a HPLC method developed in our laboratory (*3*), four new carotenoids (karpoxanthin, cucurbitaxanthin A, capsanthin 3,6-epoxide, and capsanthin 5,6-epoxide) were isolated from red paprika (*4*). The presence of these new compounds containing 3,6-epoxy- and 3,5,6-trihydroxy-end groups in the red paprika indicated new biosynthetic routes. Stuctures of carotenoids are shown in Figure 1.

In the past ten years, we reinvestigated the carotenoid composition of different kinds of paprika: *C. annuum* var. *lycopersiciforme flavum*; *C. annuum* var. *longum nigrum*; *C. annuum* var. *longum ceratoides*; and *C. annuum* var. *abbreviatum pendens* (*5*−*8*). The aim of these research projects was to study the quantitative changes of carotenoids in different varieties of paprika during ripening, and to correlate the carotenoid biosynthesis of yellow paprika to that of red paprika. As a result of these works, some minor carotenoids containing a 3,6-epoxy-end group (cucurbitaxanthin A and B, cucurbitachrome, and cycloviolaxanthin), a 3,4-didehydro-6-hydroxy-γ-end group (nigroxanthin), and a 3,5,6-trihydroxy-end group (6-epikarpoxanthin, 5,6-diepikarpoxanthin, 5,6-diepilatoxanthin, and 5,6-diepicapsokarpoxanthin) could be isolated from red paprika (*9*−*11*).

In the present paper, the carotenoid compositions of another Hungarian variety, red tomato-shaped paprika (*C. a.* var. *lycopersiciforme rubrum*), are studied. The carotenoids were obtained during the ripening of the fruits of the paprika in question. We depict a biosynthetic route in the red paprika, in comparison with that of yellow paprika.

MATERIALS AND METHODS

Materials. The fruits of pepper (*C. a.* var. *lycopersiciforme rubrum* cv. Szentesi piros paradicsom paprika) were collected from a research plantation in Szentes and were immediately transported to our laboratory. The fruits, which were at different stages of ripening, were divided into six batches according to their color, ranging from green to red. So as to obtain reliable samples, 100−300 g (fresh weight; see Table 1) of pods were used for extraction.

During our work, analytical grade chemicals were used, and authenthic samples were taken from our collection. Reports on characteristic data of the authentic minor carotenoids (cycloviolaxanthin, cucurbitaxanthin A and B, capsanthin 3,6-epoxide, 5,6-diepikarpoxanthin, 6-epikarpoxanthin, 5,6-diepilatoxanthin, 5,6-diepicapsokarpoxanthin, nigroxanthin, and capsanthone) were published earlier (*9*−*12*).

Pigment Extraction. Pigments were extracted from the samples using MeOH three times and finally diethyl ether twice. The extract was saponified in ether with 30% KOH−MeOH at room temperature. The saponified pigments were stored in benzene solution at −20 °C, under nitrogen, and away from light until the preparation of HPLC samples.

* Corresponding author. Tel: 36-72-536-000, ext. 1860. Fax: 36-72-536-225. E-mail: delijos@apacs.pote.hu.

10.1021/jf000958d CCC: $20.00
Published on Web 02/14/2001

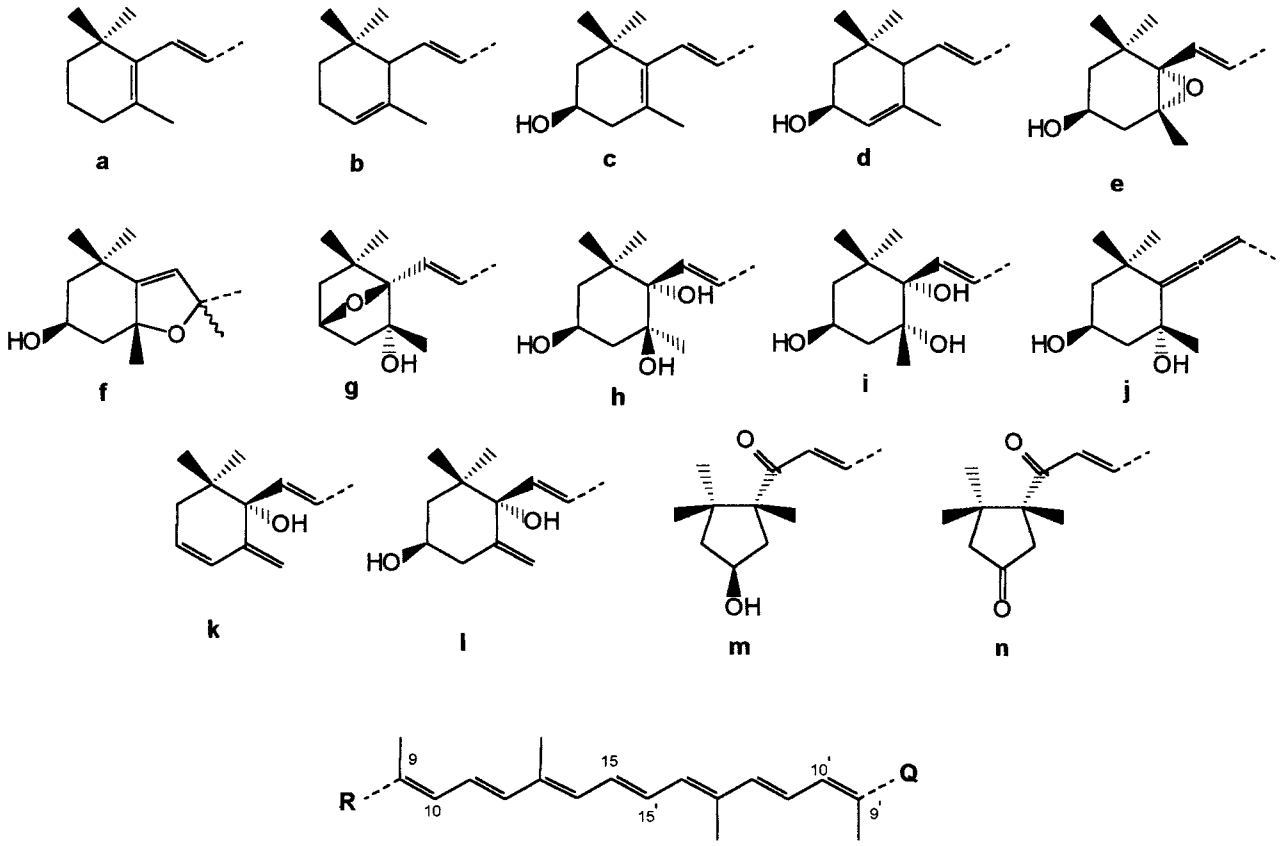

Figure 1. Structures of carotenoids. Antheraxanthin, R= e, Q = c; capsanthin, R = c, Q = m; capsanthone, R = c, Q = n; capsanthin 3,6-epoxide, R = g; Q = m; capsanthin 5,6-epoxide, R = e, Q = m; capsorubin, R = Q = m; α-carotene, R = a, Q = b; *β*-carotene, R = Q = a; cryptocapsin, R = a, Q = m; α-cryptoxanthin, R = c, Q = b; *β*-cryptoxanthin, R = c, Q = a; cucurbitaxanthin A, R = g, Q = c; cucurbitaxanthin B, R = g, Q = e; cucurbitachrome, R = g, Q = f; cycloviolaxanthin, R = Q = g; 5,6-diepicapsokarpoxanthin, R = h, Q = m; 5,6-diepikarpoxanthin, R = h, Q = c; 5,6-diepilatoxanthin, R = h, Q = e; 6-epikarpoxanthin, R = i, Q = c; lutein, R = c, Q = d; luteoxanthin, R = e, Q = f; mutatoxanthin, R = f, Q = c; neoxanthin, R = j, Q = e; nigroxanthin, R = c, Q = k; prenigroxanthin, R = c, Q = l; violaxanthin, R = Q = e; zeaxanthin, R = Q = c.

Table 1. Miscellaneous Properties

	stage of maturation					
property	green	pale green	brownish	brown	red	deep red
fresh wt of sample, g	221.40	184.21	188.74	206.80	309.50	395.13
dry wt of sample, g	10.63	8.27	9.00	7.83	11.59	12.22
dry wt/fresh wt, %	4.80	4.49	4.77	3.79	3.74	3.09
chlorophyll content, mg/100 g of dw	41.66	42.60	28.82	4.47	0.0	0.0
total carotenoid, mg/100 g of dw	19.60	33.50	64.75	230.63	542.94	1297.12
red component, %	4.7	17.0	31.1	49.8	44.2	51.4

General methods, including sample taking, extraction, workup, and quantitative determination of chlorophyll were described in detail in a previous study of yellow paprika (*5*).

High-Performance Liquid Chromatography. The chromatographic system consisted of Gynkotek pump model 300 B with Gynkotek Gradient Former, a Hewlett-Packard 1050 detector with HP ChemStation software, and a Waters-991 photodiode array detector. Columns were 250 × 4.6 mm i.d. Chromsyl C_{18}, 6 μm endcapped and Chromsyl C_{18}, 6 μm not endcapped. The eluent was 12% (v/v) H_2O in methanol (A), methanol (B), 50% (v/v) acetone in methanol (C). The gradient program was 100% A for 8 min, to 80% A/20% B in 8 min, to 50% A/50% B in 8 min, to 100% B in 7 min, 100% B 2 min, to 100% C in 6 min, 100% C 5 min (linear steps). The flow rate was 1.5 mL/min.

Identification of Peaks. The peaks in a chromatogram were identified by means of authentic carotenoid samples, different chemical tests (*3, 5*), and the UV−vis spectra of the individual peaks. Photodiode array measurements of spectral properties for the individual peaks (from 300 to 510 nm) were determined at the upslope, apex, and downslope. The matching of the three spectra indicated the degree of peak purity.

Quantification. The chromatograms were evaluated quantitatively by relating the heights of the individual carotenoids to that of canthaxanthin using an internal standard (*5*).

RESULTS AND DISCUSSION

Carotenoid Composition during Ripening. To avoid trans−cis isomerization and epoxide−furanoid oxide rearrangement, great care was taken during the isolation procedures. The individual carotenoid content and total carotenoid and chlorophyll contents were expressed on the basis of the weight and dry weight of the fruits. The different stages of ripening were characterized by the total carotenoid content of fruits (*5*).

The concentrations of the chlorophyll and carotenoid pigments were analyzed in six consecutive stages of ripeness: green, pale green, brownish, brown, red, and deep red. The changes that took place in the total carotenoid content, red carotenoid content, and chlorophyll content during ripening are shown in Table 1. The total carotenoid content was increased ca. 66-fold, and

Table 2. Relative Carotenoid Content (%) of C. Annuum Fruit at Six Stages of Maturation

			stage of maturation					
peak no.	ret. time	pigment	green	pale green	brownish	brown	red	deep red
1	10.2	5,6-diepicapsokarpoxanthin	0.35	0.16	0.36	0.32	0.22	0.32
2	10.8	5,6-diepilatoxanthin	1.74	0.60	0.91	0.60	0.14	0.28
3a	11.5		0.06	0.86	1.11	0.14	0.08	0.11
4a	11.9	λ_{max}:[b] 439, 459 nm	2.58	2.15	1.37	0.0	0.25	0.60
5a	12.9	λ_{max}:[b] 443, 462 nm	4.16	5.09	1.12	0.91	0.82	0.35
6	13.9	capsorubin	1.19	1.64	2.21	3.86	2.44	3.17
7	14.9	6-epikarpoxanthin	1.51	4.50	1.34	0.35	0.32	0.44
8	15.8	neoxanthin	3.74	5.46	3.96	0.0	0.0	0.0
9	15.8	capsanthin 5,6-epoxide	0.00	0.00	1.32	2.51	1.46	0.54
10	16.42	5,6-diepikarpoxanthin	4.16	0.20	4.78	2.88	1.22	2.76
11a	17.2	yellow mixt.	1.82	0.81	0.60	0.29	0.58	0.57
12	17.6	violaxanthin	2.20	5.45	1.08	1.65	1.63	1.92
13	18.0	capsanthin 3,6-epoxide	0.0	6.97	3.72	3.43	1.30	2.54
14	18.5	luteoxanthin 2[c]	3.62	3.35	2.05	0.56	0.83	1.11
15	19.2	luteoxanthin 1[c]	1.97	1.73	1.88	1.06	0.71	0.39
16	19.5	cucurbitaxanthin B	0.00	0.00	1.00	0.90	0.90	1.10
17	19.7	cucurbitachrome	0.00	0.00	2.00	0.78	1.50	0.71
18	21.0	capsanthin	0.24	6.60	20.95	35.08	33.70	37.02
19	21.8	capsanthone	0.00	0.00	0.10	0.37	0.40	0.44
20	22.1	antheraxanthin	1.50	2.89	2.88	2.07	0.98	0.45
21	22.7	8*S*-mutatoxanthin	0.52	0.08	0.22	0.18	0.66	1.50
22	23.6	8*R*-mutatoxanthin	0.71	1.43	1.43	1.89	2.44	2.96
23	24.4	cucurbitaxanthin A	3.44	1.94	4.67	5.88	6.36	7.25
24	25.0	9/9′-*cis*-capsanthin	0.00	0.66	1.17	1.73	1.94	3.40
25	25.6	13/13′-*cis*-capsanthin	0.00	0.93	0.98	2.84	2.45	3.83
26	26.4	lutein	31.55	17.30	5.47	0.19	0.00	0.00
27	26.4	zeaxanthin	4.11	6.31	8.65	9.26	16.54	8.16
28	27.0	nigroxanthin	0.26	0.29	0.36	0.51	0.63	0.77
29	29.0	9-*cis*-zeaxanthin	2.76	1.45	0.89	0.30	0.43	0.25
30	29.6	13-*cis*-zeaxanthin	1.65	1.35	0.53	0.39	0.47	0.33
31	29.9	15-*cis*-zeaxanthin	0.24	0.42	0.08	0.11	0.10	0.09
32a	31.0		0.21	0.36	0.28	0.31	0.43	0.51
33	33.6	cryptocapsin	0.30	0.12	0.28	0.39	0.36	0.57
34	34.9	α-cryptoxanthin	0.57	0.66	0.58	0.35	0.29	0.26
35	35.2	*β*-cryptoxanthin	0.53	1.03	2.54	3.58	5.48	3.48
36	35.5	*cis*-cryptoxanthin	0.09	0.21	0.52	0.75	0.72	0.63
37a	37.0		0.13	0.23	0.23	0.34	0.35	0.26
38	38.3	α-carotene	0.76	0.79	0.73	0.42	0.27	0.21
39	39.4	*β*-carotene	13.65	9.15	10.09	7.92	8.62	9.16
40	39.8	*cis*-*β*-carotene	0.94	1.09	1.39	1.03	0.85	0.88
	total carotenoid, mg/100 g of dw		19.60	33.50	64.75	230.63	529.94	1297.12

[a] Unidentified. [b] λ_{max} in methanol. [c] The numbers indicate the adsorption affinities, in decreasing order, on a calcium carbonate column.

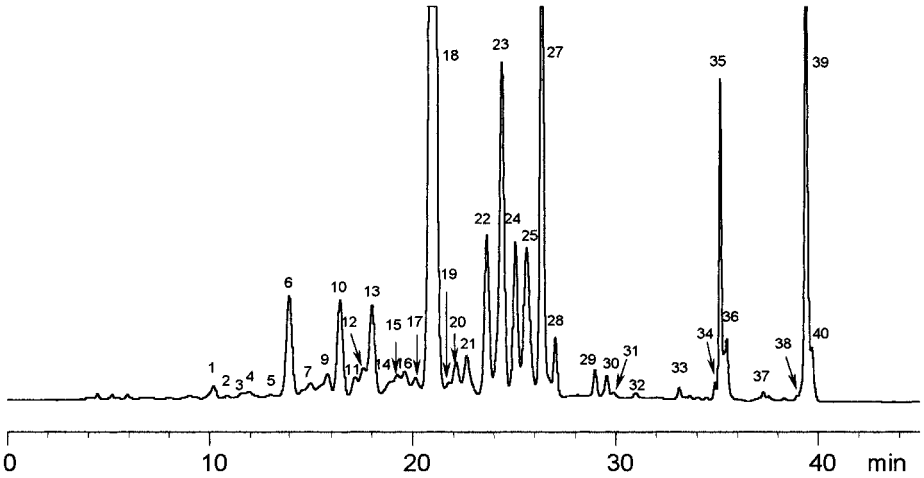

Figure 2. HPLC separation of carotenoids in ripe (deep red) paprika. Conditions: Chromsil-C_{18} 6μm endcapped, detection at 450 nm, other conditions as stated in the text. For peak numbers see Table 2.

the chlorophyll content was reduced to zero. The ratio of the red and yellow pigments increased from 0.020 to 0.65. Similar increases in pigment concentration were found by Mínguez-Mosquera and Hornero-Mendez, (*13*) and Deli et al. (*6*).

The changes in the individual carotenoid contents of fruits during ripening are given in Table 2. In all the different stages of maturation, the same 40 peaks were found (Figures 2 and 3), out of which 32 were identified. As a result of mixed peaks, 34 carotenoids were identified in the 32 peaks. The pigments were identified by means of co-chromatography, using authentic samples, various chemical tests (*14*), and UV−vis spectra.

The changes of carotenes (hydrocarbons), diols plus

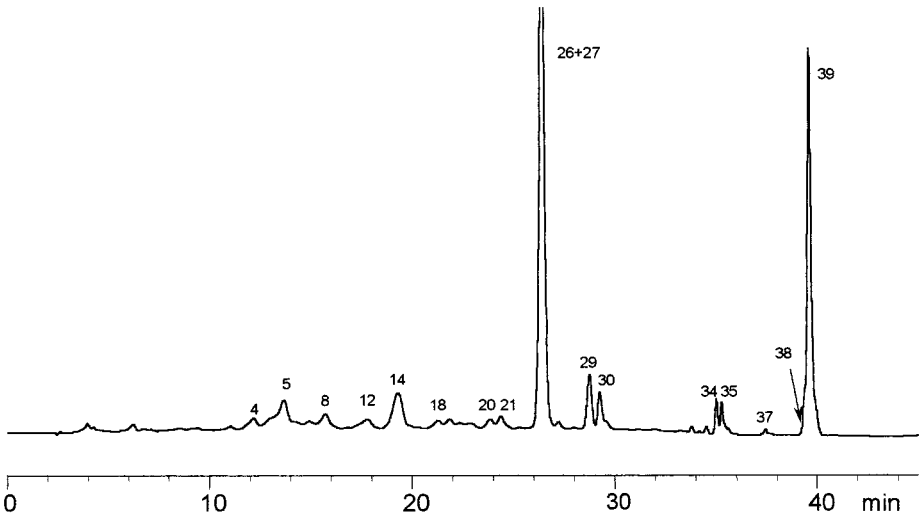

Figure 3. HPLC separation of carotenoids in unripe (green) paprika. Conditions: Chromsil-C_{18} 6μm endcapped, detection at 450 nm, other conditions as described in the text. For peak numbers see Table 2.

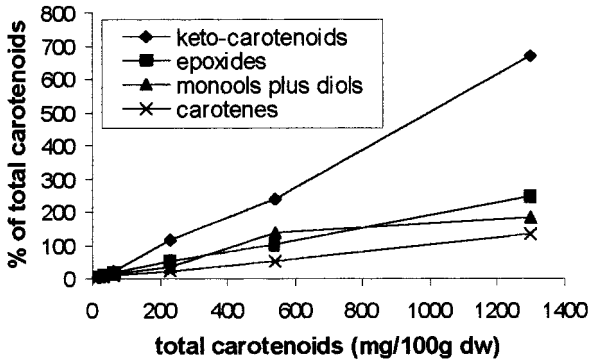

Figure 4. Pigment changes during ripening.

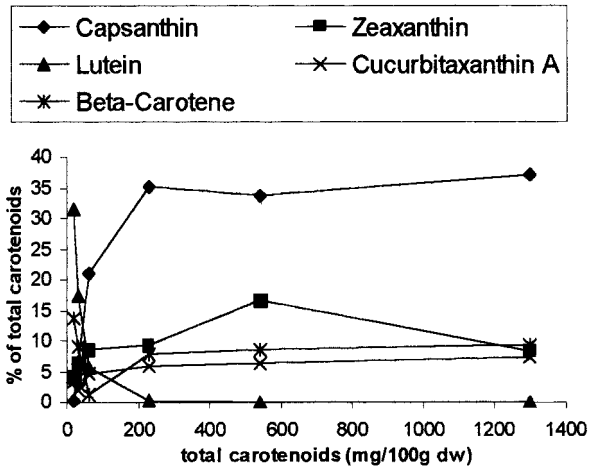

Figure 5. Changes in relative carotenoid content during ripening.

monools, epoxides, and ketocarotenoids during maturation are demonstrated in Figure 4.

The percentage distribution of carotenoids is plotted against the total carotenoid content in Figure 5. In the ripe fruits, capsanthin and zeaxanthin accounted for about 37 and 8% of the total, respectively; β-carotene and β-cryptoxanthin accounted for about 9 and 4%, respectively; and cucurbitaxanthin A accounted for about 7%. Numerous minor compounds were detected, such as violaxanthin, antheraxanthin, and capsanthin 5,6-epoxide containing a 5,6-epoxy-end group; capsanthin 3,6-epoxide and cucurbitaxanthin B containing a 3,6-epoxy-end group; 5,6-diepikarpoxanthin, 5,6-diepilatoxanthin, and 5,6-diepicapsokarpoxanthin containing a 3,5,6-trihydroxy-end group; capsanthone containing a 3-oxo-κ-end group; nigroxanthin containing a γ-end group; capsorubin, cryptocapsin, and several furanoid oxides and cis isomers.

In the unripe fruits, the main carotenoids were lutein and β-carotene. The ratio of lutein gradually decreased and finally disappeared, and in a parallel manner chlorophyll vanished too. Meanwhile, β-carotene reached a more or less constant value during ripening. In unripe paprika, until the stage of disappearance of chlorophyll, we could also detect neoxanthin, a typically chloroplast pigment.

Biosynthesis of Carotenoids. The cyclization of lycopene is the first important branch point in the pathway of carotenoid biosynthesis in higher plants. Two types of ionone rings are formed: the β-ring as an end group of both sides of β-carotene and the ϵ-ring at one side of α-carotene in addition to a β-ring (Figure 6a). The mechanism of cyclization involves proton attack at C2 and C2′ of lycopene. The resulting carbonium ion intermediate is stabilized by loss of proton from either C1 or C4 to yield a β- or ϵ-ring, respectively. The formation of the β- or ϵ-ring is under different gene control. The formation of β-carotene is catalyzed by a single enzyme, lycopene β-cyclase, whereas in the case of α-carotene, two different enzymes, lycopene β-cyclase and lycopene ϵ-cyclase, are involved (*15*). In the unripe red paprika, both of these enzymes exist, and β-carotene and lutein as main carotenoid are formed. During ripening the enzyme activity of ϵ-cyclase decreases and finally leaves off, and meanwhile, the lutein disappears too. In contrast, in the extract of yellow paprika (*C. annuum* var. *lycopersiciforme flavum* cv. Szentesi sárga paradicsom paprika) investigated earlier, we always could detect carotenoids containing an ϵ-ring such as α-carotene, α-cryptoxanthin, lutein, and lutein 5,6-epoxide (*5*): consequently, in this variety, the ϵ-cyclase exists during ripening. The lycopene β-cyclase has been isolated from a variety of red paprika (*16*, *17*) and cloned, but the ϵ-cyclase have not been isolated from paprika until now.

Xanthophylls are enzymically formed oxidation products of α- and β-carotene. The most common oxygen groups found in paprika xanthophylls are hydroxy at

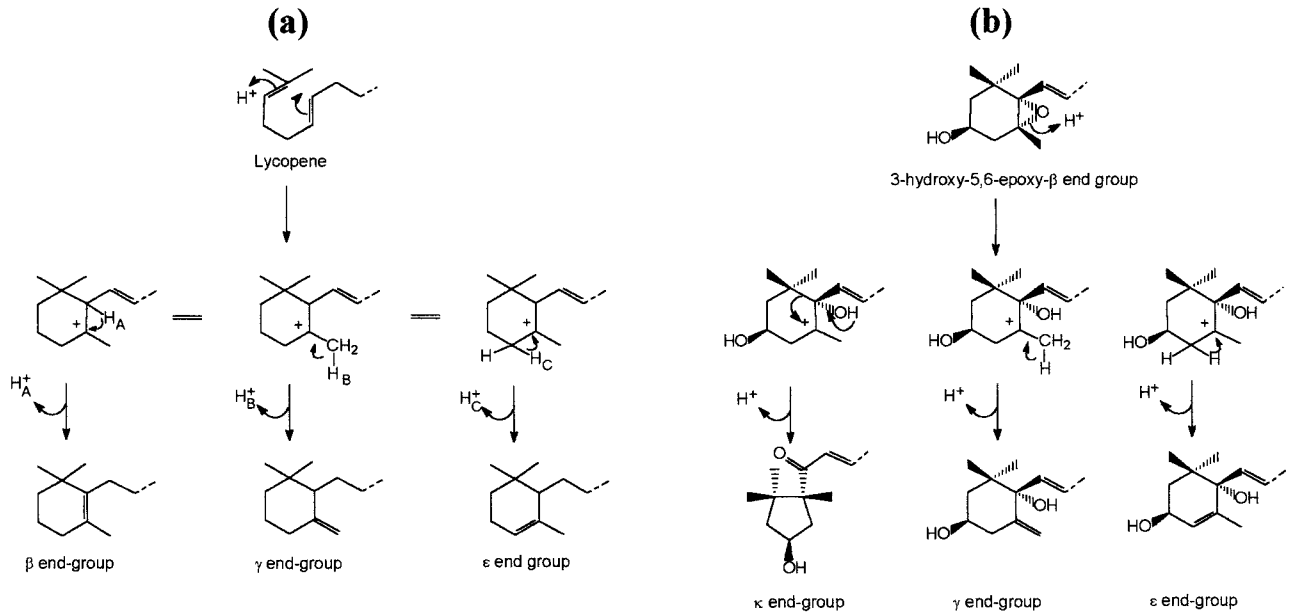

Figure 6. Comparison of the proposed mechanisms for lycopene cyclization (a) and transformations of the 3-hydroxy-5,6-epoxy group (b).

C3, epoxy at the 5,6 position of the ionone ring, and the keto-group.

Zeaxanthin and lutein are formed by the hydroxylation of the 3 and 3′ carbon atoms of β,β-carotene or β,ϵ-carotene, respectively, by separate hydroxylases specific for the β and ϵ rings (*18*−*20*). Starting from zeaxanthin, antheraxanthin with one epoxy group and the diepoxy-violaxanthin are formed by the introduction of these epoxy groups into the 5,6 and 5′,6′ position. The enzymes involved in these reactions are assumed to be mixed function oxygenases. Bouvier et al. (*21*) found that the 3-hydroxy-β rings of zeaxanthin and antheraxanthin were substrates for the paprika enzyme, but, surprisingly, the 3-hydroxy-β ring of lutein was not epoxidated in vitro. In accordance with this result, we have not detected lutein 5,6-epoxide in our studies on different varieties of red paprika (*6*−*8*). On the other hand, in the yellow paprika (*C. annuum* var. *lycopersiciforme flavum* cv. Szentesi sárga paradicsom paprika), the lutein 5,6-epoxide seems to be an end product of the carotenoids containing ϵ ring (*5*).

In an earlier work (*7*), we have depicted the possible biosynthetic transformation of the 5,6-epoxy end group in paprika. It could be seen from this scheme that five different biosynthetic routes exist, out of which, pinacol rearrangement into a κ-end group is the most important. Furthermore, the 5,6-epoxy end group may undergo allenic rearrangament, epoxide ring opening, endo epoxide rearrangement, and furanoid-oxide arrangement resulting in 3,5-dihydroxy-allenic-, 3,5,6-trihydroxy-β-, 3,6-epoxy-β-, and 5,8-epoxy-end groups, respectively.

In the unripe red paprika and in all the different stages of maturation of yellow paprika, the epoxy-carotenoid neoxanthin with an allenic-end group is present. It has been assumed that this allenic group originates from the 5,6-epoxy-5,6-dihydro-β rings of violaxanthin (*22*). This reaction is catalyzed by neoxanthin synthase (NES) which has not been isolated and characterized.

As a result of our paprika analyses, we have isolated some minor compounds with 3,5,6-trihydroxy-β-end groups. It is of great interest that for 5,6-diepikarpoxanthin, 5,6-diepilatoxanthin, and 5,6-diepikarpoxanthin isolated from red paprika, the 3*S*,5*S*,6*S*-configuration, and for 6-epikarpoxanthin isolated from red paprika, the 3*S*,5*R*,6*S*-configuration, were established (*11*). In our opinion, in the red paprika, the ring opening of the carotenoid 5,6-epoxide and the formation the 3,5,6-trihydroxy-carotenoids may be connected to the formation of the κ-end group.

Recently, the capsanthin-capsorubin synthase (CCS), an enzyme catalyzing the conversion of a 5,6-epoxy-end group into a κ-end group, was isolated and characterized (*16*). Certain similarities to the *C. annuum* lycopene cyclase, the enzyme catalyzing the cyclization of lycopene, were observed (*23*). The fact that CCS also exhibits lycopene cyclase activity is likely to be related to the similarities in the chemical mechanisms leading to the formation of β-rings in β-carotene and κ-rings in capsanthin and capsorubin. In both mechanisms a carbenium ion at C(5) as intermediate is formed (*17*). In addition, both reactions are likely to be initiated by a protonic attack on either a double bond or an epoxy group (Figure 6).

On the basis of the above-described reaction mechanism for CCS, we suggest a new mechanism for the formation of 3,5,6-trihydroxy-carotenoids isolated from red paprika. Either the (3*S*,5*S*,6*S*)- or the (3*S*,5*R*,6*S*)-end group may be formed via the carbenium ion at C5. Therefore, during the enzyme-catalyzed hydrolysis of carotenoid-5,6-epoxides, the configuration at C5 may change, and the configuration at C6 remains unchanged (See: 5,6-diepikarpoxanthin and 6-epikarpoxanthin). We have previously described the isolation of 5,6-diepikarpoxanthin and 5,6-diepicapsokarpoxanthin, both with the (3*S*,5*S*,6*S*)-3,5,6-trihydroyxy-β end-group, and 6-epikarpoxanthin, which contains the (3*S*,5*R*,6*S*)-3,5,6-trihydroxy-β end group, from petals of *Lilium tigrinum* (*24*) and the isolation of 5,6-diepikarpoxanthin from the fruits of *Asparagus falcatus* (*25*). Our investigations revealed that in plants containing carotenoids with the κ end-group and carotenoids with the 3,5,6-trihydroxy-β end-group, the chirality of the latter end group was always the same, i.e., 3*S*,5*S*,6*S* and 3*S*,5*R*,6*S*, irrespec-

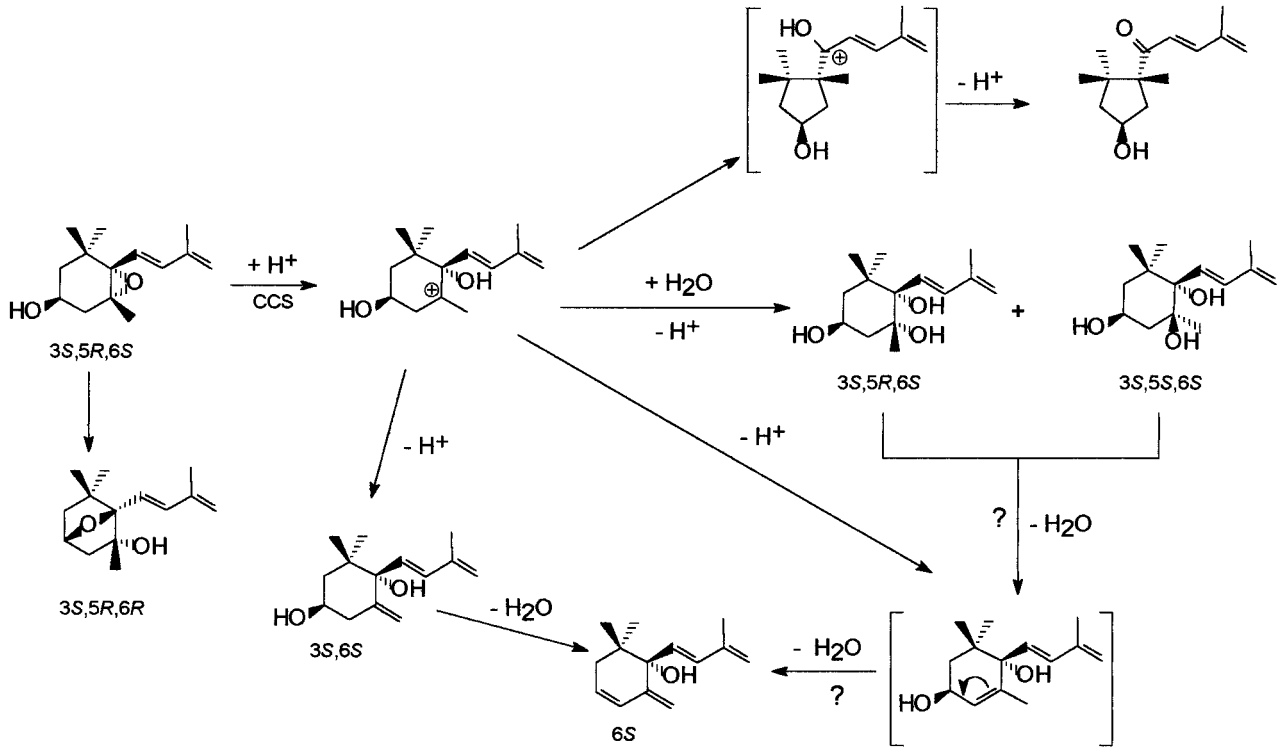

Figure 7. The possible formation of a variety of carotenoid end groups from a 3-hydroxy-5,6-epoxy-5,6-dihydro-β-ring.

tive of the natural source, as examplified by the isolation of these carotenoids from paprika, *Lilium tigrinum*, and *Asparagus falcatus*. It should be noted that until now it has not been clarified whether the 3,5,6-trihydroxy-carotenoids are intermediates or byproducts of pinacol rearrangement. The clearing up of this question demands further biochemical investigations which are in progress.

During our investigations of different species of paprika (*Capsicum annuum*) some novel carotenoids with the 7-oxabicyclo[2.2.1]heptyl (3,6-epoxycyclohexyl) end group such as cucurbitaxanthin A and B, capsanthin-3,6-epoxide, and cycloviolaxanthin have been isolated and characterized (*10*). Earlier we supposed that in the probable biosynthetic route of the formation of a 3,6-epoxy-end group from a 5,6-epoxy ring, the 3,5,6-trihydroxy compounds may occur as intermediates. Now, we have to revise this assumption. Under the acid-catalyzed hydrolysis of carotenoid 5,6-epoxides (capsanthin 5,6-epoxide and antheraxanthin) the formation of capsanthin 3,6-epoxide and cucurbitaxanthin A were observed (*26*, *27*). The formation of 3,6-epoxy carotenoids in the acid-catalyzed hydrolysis has not been reported previously. Both the acid-catalyzed hydrolysis of carotenoid 5,6-epoxides and the furanoid-oxide arrangement involve the formation of an carbenium cation at C6 (*26*, *28*). The nucleophilic attack of the 3-hydroxy group on the C6 results in the 3,6-epoxy-end group. Although in the yellow paprika we could not detect the carotenoid 3,6-epoxides, we assume that in the red paprika a dehydratase enzyme must exist which catalyzed this transformation. In Table 2 it is demonstrated that furanoid oxides (luteoxanthin and mutatoxanthin) are always present during the process of ripening. The ratio of mutatoxanthin epimers displays considerable difference from the end product of the acid treatment of antheraxanthin under laboratory conditions. The ratio of 8*R*-mutatoxanthin to 8*S*-mutatoxanthin was constant in yellow pepper during maturation (*5*, *29*). Therefore, we assume that the 8*S*-mutatoxanthin and some of the 8*R*-mutatoxanthin may have been post-mortem artifacts in the red paprika. However, a stereospecific enzymatic action linked to the endo-epoxide rearrangement of antheraxanthin to cucurbitaxanthin A cannot be ruled out in the formation of 8*R*-mutatoxanthin.

From the extracts of different varieties of red paprika, we have always detected and isolated nigroxanthin, which contained a 6-hydroxy-3,4-didehydro-γ end-group (*6*−*9*). Recently, we have isolated and characterized an other new carotenoid, prenigroxanthin, which contained a 3,6-dihydroxy-γ end-group (*30*). However, the configuration of the 6-hydroxy-group remained unknown in both carotenoids containing a γ-end group. These compounds may be formed from antheraxanthin and their occurrence is interrelated with the biosynthesis of the κ end-group. Following is the probable biosynthetic route of the formation of the γ end-group: the enzymatic opening of the 5,6-epoxy ring results in a carbenium ion at C5 in compliance with Figure 6b. This intermediate can be stabilized in three different routes: (a) a κ end-group is formed; (b) a 3,6-dihydroxy-γ end-group is formed; or (c) a 3,6-dihydroxy-ϵ end-group is formed. We assume that the later end group is not stable and it can be easily arranged to a 6-hydroxy-3,4-didehydro-γ end group by water elimination. In these reactions, the configuration at C6 remains unchanged, so the proposed structures with the 6*S* configuration for nigroxanthin and prenigroxanthin are strongly supported. The plausible biosynthetic transformations of the 5,6-epoxy-β end-group are summarized in Figure 7.

ACKNOWLEDGMENT

We gratefully acknowledge the gifts of paprika from Dr. H. Darázsi (Research Institute of Seed Production and Trading Co., Szentes, Hungary). We are grateful to Prof. G. Britton (Liverpool) for helpful discussions.

LITERATURE CITED

(1) Cholnoky, L.; Györgyfy, K.; Nagy, E.; Pánczél, M. Investigations of carotenoid pigments. I. Pigments of red paprika. *Acta Chim. Acad. Sci. Hung.* **1955**, *6*, 143−147.

(2) Cholnoky, L.; Györgyfy, K.; Nagy, E.; Pánczél, M. Investigations of carotenoid pigments. II. Pigments of the yellow paprika. *Acta Chim. Acad. Sci. Hung.* **1958**, *16*, 227−246.

(3) Baranyai, M.; Matus, Z.; Szabolcs, J. Determination by HPLC of carotenoids in paprika products. *Acta Aliment.* **1982**, *11*, 309−323.

(4) Parkes, K. E. B.; Pattenden, G.; Baranyai, M.; Molnár, P.; Szabolcs, J.; Tóth, G. Novel carotenoid 3,6-epoxides from red paprika (*Capsicum annuum*). *Tetrahedron Lett.* **1986**, *27*, 2535−2537.

(5) Matus, Z.; Deli, J.; Szabolcs, J. Carotenoid composition of yellow pepper during ripening: Isolation of β-cryptoxanthin 5,6-epoxide. *J. Agric. Food Chem.* **1991**, *39*, 1907−1914.

(6) Deli, J.; Matus, Z.; Szabolcs, J. Carotenoid composition in the fruits of black paprika (*Capsicum annuum* var. *longum nigrum*) during ripening. *J. Agric. Food Chem.* **1992**, *40*, 2072−2076.

(7) Deli, J.; Matus, Z.; Tóth, G. Carotenoid composition in the fruits of *Capsicum annuum* cv. Szentesi Kosszarvú during ripening. *J. Agric. Food Chem.* **1996**, *44*, 711−716.

(8) Deli, J.; Tóth, G. Carotenoid composition in the fruits of *Capsicum annuum* cv. Bovet 4 during ripening. *Z. Lebensm. Unters. Forsch. A* **1997**, *205*, 388−391.

(9) Deli, J.; Matus, Z.; Molnár, P.; Tóth, G.; Szalontai, G.; Steck, A.; Pfander, H. Nigroxanthin (3′,4′-didehydro-β,γ-carotene-3,6′-diol) a new carotenoid isolated from paprika (*Capsicum annuum* var. *longum nigrum*). *Chimia* **1994**, *48*, 102−104.

(10) Deli, J.; Molnár, P.; Matus, Z.; Tóth, G.; Steck, A. Reisolation of carotenoid 3,6-epoxides from red paprika. *Helv. Chim. Acta* **1996**, *79*, 1435−1442.

(11) Deli, J.; Matus, Z.; Molnár, P.; Tóth, G.; Steck, A.; Pfander, H. Isolation of carotenoids with 3,5,6-trihydroxy-5,6-dihydro-β-end groups from red paprika (*Capsicum annuum*). *Helv. Chim. Acta* **1998**, *81*, 1233−1241.

(12) Deli, J.; Matus, Z.; Molnár, P.; Tóth, G.; Steck, A.; Pfander, H. Isolation of capsanthone ((*all*-E,3*R*,5′*R*)-3-hydroxy-β,κ-caroten-3′,6′-dion) from paprika (*Capsicum annuum*). *Chimia* **1995**, *49*, 69−71.

(13) Mínguez−Mosquera, M. I.; Hornero−Mendez, H. Formation and transformation of pigments during the fruit ripening of *Capsicum annuum* cv. Bola and Agridulce. *J. Agric. Food Chem.* **1994**, *42*, 38−44.

(14) Matus, Z.; Baranyai, M.; Tóth, Gy.; Szabolcs, J. Identification of oxo, epoxy and some *cis*- carotenoids in high performance liquid chromatography. *Chromatographia* **1981**, *14*, 337−340.

(15) Sandmann, G. Carotenoid biosynthesis in microorganisms and plants. *Eur. J. Biochem.* **1994**, *223*, 7−24.

(16) Bouvier, F.; Huguney, P.; d'Harlingue, A.; Kuntz, M.; Camara, B. Xanthophyll biosynthesis in chromoplasts: isolation and molecular cloning of an enzyme catalyzing the conversion of 5,6-epoxy-carotenoid into keto-carotenoid. *Plant J.* **1994**, *6*, 45−54.

(17) Bouvier, F.; d'Harlingue, A.; Camara, B. Molecular analysis of carotenoid cyclase inhibition. *Arch. Biochem. Biophys.* **1997**, *346*, 53−64.

(18) Pogson, B.; McDonald, K. A.; Truong, M.; Britton, G.; DellaPenna, D. *Arabidopsis* carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. *Plant Cell* **1996**, *8*, 1627−1639.

(19) Sun, Z. R.; Gantt, E.; Cunningham, F. X., Jr. Cloning and functional analysis of the β-carotene hydroxylase of *Arabidopsis thaliana*. *J. Biol. Chem.* **1996**, *271*, 24349−24352.

(20) Bouvier, F.; Keller, Y.; d'Harlingue, A.; Camara, B. Xanthophyll biosynthesis: molecular and functional characterization of carotenoid hydroxylases from pepper fruits (*Capsicum annuum* L.). *Biochim. Biophys. Acta* **1998**, *1391*, 320−328.

(21) Bouvier, F.; d'Harlingue, A.; Hugueney, P.; Marin, E.; Marion-Poll, A.; Camara, B. Xanthophyll biosynthesis cloning, expression, functional reconstitution, and regulation of β-cyclohexenyl carotenoid epoxidase from pepper (*Capsicum annuum*). *J. Biol. Chem.* **1996**, *271*, 28861−28867.

(22) Spurgeon, S. L.; Porter, J. W. Biosynthesis of Carotenoids. In *Biochemistry of Isoprenoid Compounds;* Porter, J. W., Spurgeon, S. L., Eds.; John Wiley and Sons: New York, 1980; pp 1−122.

(23) Hugueney, P.; Badillo, A.; Chen, H.; Klein, A.; Hirschberg, J.; Camara, B.; Kuntz, M. Metabolism of cyclic carotenoids: A model for the alteration of this biosynthetic pathway in *Capsicum annuum* chromoplasts. *Plant J.* **1995**, *8*, 417−424.

(24) Deli, J.; Molnár, P.; Matus, Z.; Tóth, G.; Steck, A.; Pfander, H. Isolation and characterization of 3,5,6-trihydroxy-carotenoids from petals of *Lilium tigrinum*. *Chromatographia* **1998**, *48*, 27−31.

(25) Deli, J.; Molnár, P.; Ösz, E.; Tóth, G. Analysis of the carotenoids of the fruits of *Asparagus falcatus*: Isolation of 5,6-diepikarpoxanthin. *Chromatographia* **2000**, *51*, S183−187.

(26) Deli, J.; Matus, Z.; Molnár, P.; Tóth, G.; Steck, A.; Pfander, H. Partial synthesis and characterization of capsokarpoxanthins and 3,6-epoxycapsanthins. *Helv. Chim. Acta* **1998**, *81*, 1242−1253.

(27) Molnár, P.; Deli, J.; Matus, Z.; Tóth G.; Steck, A.; Pfander, H. Partial synthesis and characterization of karpoxanthins and cucurbitaxanthin A epimers. *Helv. Chim. Acta* **1999**, *82*, 1994−2002.

(28) Buchecker, R.; Marti, U.; Eugster, C. H. Syntheses of optically active carotenoids with 3,5,6-trihydroxy-5,6-dihydro β-end groups. *Helv. Chim. Acta* **1984**, *67*, 2043−2056.

(29) Molnár, P.; Deli, J.; Matus, Z.; Tóth, G.; Steck, A.; Pfander, H. Isolation of mutatoxanthins from red Paprika. *Eur. Food Res. Technol.* **2000**, *211*, 396−399.

(30) Deli, J.; Molnár, P.; Tóth, G.; Traber, B.; Pfander, H. "Prenigroxanthin" ((all-E,3R,3′S,6′S)-β,γ-carotene-3,3′6′-triol) a novel carotenoid from red paprika (*Capsicum annuum*). *Tetrahedron Lett.* **2001**, *42*, 1395−1397.

Received for review July 31, 2000. Revised manuscript received December 29, 2000. Accepted December 29, 2000. This study was supported by a grant from the Hungarian Ministry of Health (ETT 333/2000).

JF000958D