Carotenoid Composition in the Fruits of *Asparagus officinalis*

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The carotenoid pigments of the ripe and unripe fruits of *Asparagus officinalis* were investigated by means of an HPLC technique. Capsanthin, capsorubin, capsanthin 5,6-epoxide, antheraxanthin, violaxanthin, neoxanthin, mutatoxanthin epimers, zeaxanthin, lutein, β-cryptoxanthin, β-carotene, and some cis isomers were found. Carotenoids with 3,5,6-trihydroxy and 3,6-epoxy β-end groups could not be detected.

Keywords: *Asparagus officinalis; carotenoids; analysis; biosynthesis*

INTRODUCTION

Capsanthin, which was first isolated from the red fruit of *Capsicum annuum* (Zechmeister and Cholnoky, 1927), has also been found in the pollen anthers of *Lilium tigrinum* (Karrer and Oswald, 1935) and *Aesculus hippocastanum* (Neamtu et al., 1969), in the fruit of *Berberis* spp. (Bubicz, 1965) and *Asparagus officinalis* (Simpson et al., 1977), and in *Lilium amabile* petals (Seybold, 1953). Capsorubin has been isolated from the integument of *Encephalartos altensteinil*, the petals of *Cajophora lateritia* (Seybold, 1953), the pollen anthers of *A. hippocastanum* (Neamtu et al., 1969), and the fruits of *C. annuum* (Davies et al., 1970) and *A. officinalis* (Simpson et al., 1977).

During our investigations of different species of paprika (*C. annuum*) (Deli et al., 1992, 1996a, 1997), some novel carotenoids with a 3,6-epoxy β-end group (Deli et al., 1996b) and a 3,5,6-trihydroxy β-end group were isolated and characterized (Deli et al., 1998). These compounds may be formed from antheraxanthin and violaxanthin, and their biosynthesis may be interrelated with that of capsanthin and capsorubin, though this has not been proved experimentally.

Simpson et al. (1977) investigated the carotenoid composition in the fruits of *A. officinalis*. In the ripe fruits, the principal pigments were capsanthin, β-carotene, and zeaxanthin, and there were also other pigments in small amounts such as capsorubin, cryptoxanthin, cryptocapsin, antheraxanthin, violaxanthin, and capsanthin isomers. Since both chromoplast structure and the main component of carotenoids are similar in paprika as well as in asparagus fruits (Simpson et al., 1977), we assumed that the biosynthesis of carotenoids proceeds in a similar way in both plants. Therefore, we decided to reinvestigate the carotenoid composition in asparagus fruits, paying special attention to the formation of minor carotenoids. In this paper, we describe the HPLC study of the carotenoid composition in the fruits of *A. officinalis*.

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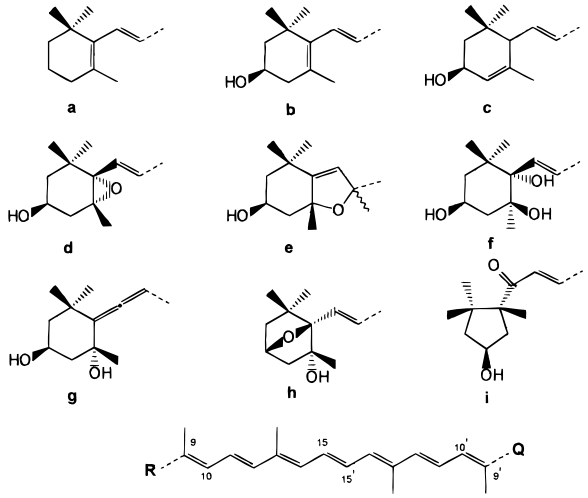
Chart 1[a]



[a] Antheraxanthin, R = d, Q = b; auroxanthin, R = Q = e; capsanthin, R = b, Q = i; capsanthin 3,6-epoxide, R = h; Q = i; capsanthin 5,6-epoxide, R = d, Q = i; capsochrome, R = e, Q = i; capsorubin, R = Q = i; β-carotene, R = Q = a; cryptocapsin, R = a, Q = i; β-cryptoxanthin, R = b, Q = a; cucurbitaxanthin A, R = h, Q = b; cucurbitaxanthin B, R = h, Q = d; cycloviolaxanthin, R = Q = h; 5,6-diepikarpoxanthin, R = f, Q = b; 5,6-diepilatoxanthin, R = f, Q = d; 5,6-diepicapsokarpoxanthin, R = f, Q = i; lutein, R = b, Q = c; luteoxanthin, R = d, Q = e; mutatoxanthin, R = e, Q = b; neoxanthin, R = g, Q = d; violaxanthin, R = Q = d; zeaxanthin, R = Q = b.

MATERIALS AND METHODS

General methods, including sample taking, extraction, and workup, were described in detail in a previous study (Matus et al., 1991).

Materials. The fruits of *A. officinalis* were obtained from a commercial garden in Pécs (southern Hungary). The fruits were divided into three groups according to their color: green, brown, and red (Minguez-Mosquera and Hornero-Mendez, 1994; Márkus et al., 1999; Deli et al., 1992, 1996a, 1997; Simpson et al., 1977). Analytical grade chemicals were used, and authentic samples were taken from our collection. Reports on characteristic data of the authentic minor carotenoids extracted from red paprika (cucurbitaxanthin A, capsanthin 3,6-epoxide, capsanthin 5,6-epoxide, 5,6-diepikarpoxanthin) were published earlier (Deli et al., 1996b, 1998; Parkes et al., 1986).

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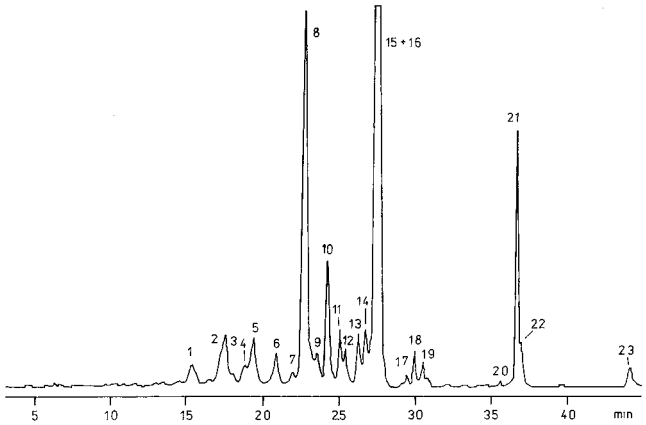

Figure 1. HPLC separation of carotenoids in ripe (red) asparagus fruits. Conditions: Chromsil C_{18}, 6 μm, endcapped; detection at 450 nm; other conditions as in the text. For peak numbers, see Table 1.

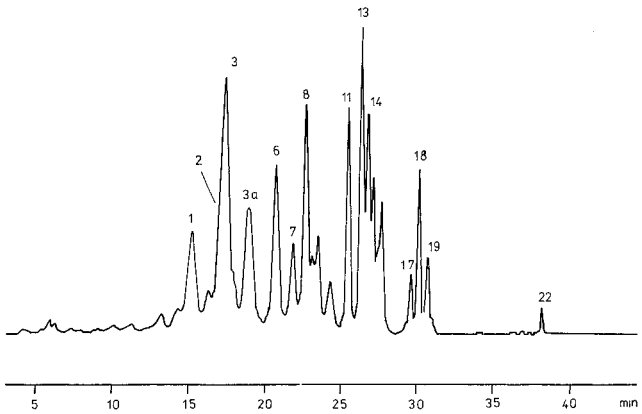

Figure 2. HPLC separation of carotenoids in fraction 1. Conditions: Chromsil C_{18}, 6 μm, endcapped; detection at 450 nm; other conditions as in the text. For peak numbers, see Table 1.

Pigment Extraction. Pigments were extracted from a sample (10−40 g), first by 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 were kept away from light until the preparation of HPLC samples.

High-Performance Liquid Chromatography. The chromatographic system consisted of a Gynkotek pump model 300 B with a Gynkotek gradient former 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), or 50% (v/v) acetone in methanol (C). The gradient program was 100% A 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 cm^3/min.

Identification of Peaks. Carotenoids were identified according to their chromatographic behavior on HPLC and UV−vis absorption spectra, by comparing both their retention time and the absorption spectra with those of authentic carotenoids. Examination of the pigment functional groups was carried out using specific chemical tests: conversion of the 5,6-epoxide group into a 5,8-furanoid in acidic medium and reduction with sodium borohydride of ketone groups (Matus et al., 1991; Baranyai et al., 1982). Photodiode array measurements of spectral properties for the individual peaks (from 300 to 500 nm) were determined at the upslope, apex, and downslope. The matching of the three spectra indicated the degree of peak purity.

Quantification. The quantitative determination of the total carotenoid content of fruits was performed by UV−vis (Davies, 1976). The chromatograms were evaluated quantitatively by relating the areas of the individual carotenoids.

Column Chromatography. The extract of ripe fruits was chromatographed on 1 column (3 × 30 cm, $CaCO_3$; Biogal, Hungary) with hexane−benzene (1:1) as developing solvent. After development four fractions were visible: fraction 1, 8 mm, brick red band; fraction 2, 15 mm, pink band; fraction 3, 10 mm, pale yellow band; fraction 4, 8 mm, yellow band. After processing (extruding the column, cutting the column into fractions, and extracting with MeOH), the composition of the fractions was monitored by HPLC.

RESULTS AND DISCUSSION

The identification of carotenoids was based on their HPLC retention times, UV−vis spectra, and, when possible, cochromatography with authentic samples.

The HPLC chromatogram of the saponified extract of ripe fruits is shown in Figure 1. It is easy to see that the main carotenoids are capsanthin (peak 8), zeaxanthin and lutein (peak 15+16), and β-cryptoxanthin (peak 21).

To obtain full evidence for the missing minor carotenoids containing 3,6-epoxy and 3,5,6-trihydroxy end groups, the whole extract of ripe fruits was separated by column chromatography ($CaCO_3$) with a mixture of benzene−hexane to give four fractions containing carotenoids with different polarities.

The HPLC chromatogram of fraction 1, the most complex mixture, is shown in Figure 2. In fraction 1, 18 peaks were detected of which 13 could be identified. The main carotenoids were the 9/9′- and 13/13′-*cis*-capsanthins (peaks 13 and 14) which showed a single broad band at 464 and 461 nm and the cis peaks at 362 and 356 nm, respectively. The next major peak at 15.5 min was a mixed peak. The upslope of this peak displayed UV−vis λ_{max} at 464, 436, and 414 nm which is characteristic of neoxanthin. The apex and downslope showed λ_{max} at 465 nm, without fine structure, which is typical of capsanthin 5,6-epoxide. A similar effect was observed in the case of Kosszarvu paprika (*C. annuum* var. *longum ceratoides*) and Bovet 4 paprika (*C. annuum* var. *abbreviatum pendens*) (Deli et al., 1996; Deli and Tóth, 1997), when the neoxanthin and capsanthin 5,6-epoxide showed the same retention time. While in the different kinds of red paprika the neoxanthin appeared only at the unripe stage, in the asparagus fruits the neoxanthin occurred at the ripe stage also.

In this fraction the capsochrome (furanoid oxide of capsanthin 5,6-epoxide showed λ_{max} at 447 nm without fine structure) could be detected (peak 3A), which was covered by the peak of violaxanthin in the chromatogram of the full extract (Figure 1). (8*R*)-Mutatoxanthin was found at 25.4 min (λ_{max}: 451, 426 nm). The occurrence in fraction 1 and the chromatographic behavior of (8*R*)-mutatoxanthin epimer were in full agreement with that found earlier (Molnár et al., 2000). Two further furanoid oxides were detected, namely, one of the luteoxanthin (λ_{max}: 447, 422 nm) and auroxanthin epimers.

Several other cis compounds were also detected in fraction 1. The characteristic triple peaks (Matus et al., 1991) could be found at 30-min retention time (9/9′-*cis*-lutein, 13/13′-*cis*-lutein + 9-*cis*-zeaxanthin, and 13-*cis*-zeaxanthin), and peak 22 was identified as *cis*-β-cryptoxanthin.

In fraction 1, the identification of the most important 13 peaks was performed in the HPLC chromatogram, but 5 peaks remained unidentified. Using authentic

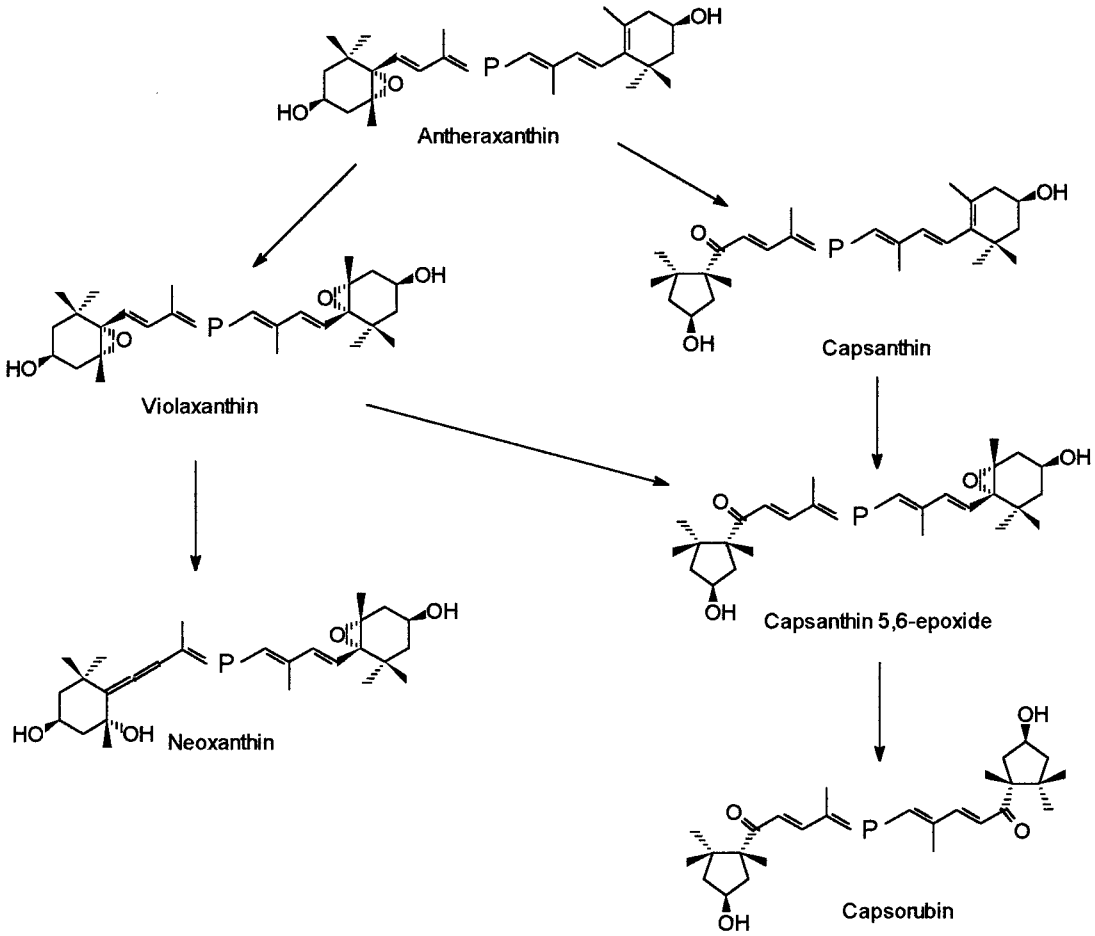

Figure 3. Possible transformation of antheraxanthin in asparagus fruits.

samples an attempt was made to identify 5,6-diepikarpoxanthin (carotenoid with a 3,5,6-trihydroxy β-end group) and capsanthin 3,6-epoxide (carotenoid containing a 3,6-epoxy β-end group). The cochromatography of fraction 1 with the authentic samples resulted in new peaks, which showed the absence of these carotenoids.

One of the major carotenoids was found in fraction 2 at 22.7 min. According to its UV−vis spectra (469 nm) and cochromatography with authentic sample (extracted from red paprika) the carotenoid was identified as capsanthin.

In fraction 3, three peaks could be separated by HPLC. The main peak at 24.2 min was identified as antheraxanthin. This compound displayed UV−vis λ_{max} at 472 and 443 nm in HPLC eluent (6% H_2O in methanol). After acidic treatment the peak disappeared in the chromatogram and a new peak appeared at 25.4 min (λ_{max}: 451, 426 nm), and at the same time the peak at 25.1 min (λ_{max}: 451, 426 nm) became stronger. In the UV−vis spectra, a hypsochromic shift of 20 nm was observed after the acidic treatment indicating the presence of one 5,6-epoxide group in the primary carotenoid (Eugster, 1995). Cochromatography with the authentic antheraxanthin confirmed that this carotenoid was identical to antheraxanthin.

The second peak (at 25.1 min) in fraction 3 was identical to one of the mutatoxanthin epimers which were formed under acidic treatment. It has previously been found in red paprika (Molnar et al., 2000), and this compound was identified as (8*S*)-mutatoxanthin. The third peak at 19.3 min was identified as violaxanthin. Carotenoid showed λ_{max} at 468, 440, and 414 nm, with significant fine structure, in the HPLC eluent (~9% H_2O in methanol). After acidic treatment, the UV−vis spectrum showed a ~40 nm hypsochromic shift indicating two 5,6-epoxy end groups (Eugster, 1995). Cochromatography with the authentic sample (extract from yellow paprika) confirmed the identity with violaxanthin.

Fraction 4 contained the least polar carotenoids. The main peak was a mixed peak of zeaxanthin and lutein.

Table 1. Relative Carotenoid Content (%) of Asparagus Fruit at Three Stages of Maturation

peak no.	t_R (min)	pigment	green	brown	red
1	15.5	capsorubin	5.22	2.91	1.65
2	17.1	neoxanthin	9.17	8.10	0.87
3	17.5	capsanthin 5,6-epoxide			2.80
4	18.8	unidentified mixture			0.94
5	19.3	violaxanthin	3.57	2.28	2.80
6	20.8	luteoxanthin	2.17	1.82	1.81
7	21.9	auroxanthin	1.31	2.50	0.50
8	22.7	capsanthin	3.82	11.89	18.64
9	23.6	unidentified mixture	4.97	4.49	1.34
10	24.2	antheraxanthin	1.81	1.74	5.30
11	25.1	(8*S*)-mutatoxanthin	0.17	0.93	2.00
12	25.4	(8*R*)-mutatoxanthin	0.21	0.96	1.43
13	26.3	9/9′-*cis*-capsanthin		0.66	1.96
14	26.7	13/13′-*cis*-capsanthin		0.74	2.25
15	27.3	lutein	40.91	34.38	14.78
16	27.6	zeaxanthin			27.02
17	29.5	9/9′-*cis*-lutein	1.62	1.12	0.36
18	29.9	13′/13′-*cis*-lutein + 9-*cis*-zeaxanthin	2.63	2.66	1.10
19	30.5	13-*cis*-zeaxanthin			1.00
20	35.6	cryptocapsin	0.38	1.48	0.08
21	36.6	β-cryptoxanthin	1.00	1.33	6.34
22	37.0	*cis*-β-cryptoxanthin	0.76	0.06	1.29
23	44.2	β-carotene	16.48	14.52	0.82
total carotenoid (μg/g fresh wt)			22	25	123

These carotenoids could be separated and identified on a not endcapped solid phase in accordance with previous reports (Matus et al., 1991). The next major peak in this fraction at 36.6 min was identified as β-cryptoxanthin. Carotenoid showed λ_{max} at 478 and 452 nm in the HPLC eluent (~25% acetone in methanol). Cochromatography with the authentic sample confirmed the identity with β-cryptoxanthin. The last small peak at 44.2 min was identified as β-carotene by cochromatography with anauthentic sample.

The HPLC chromatogram of green asparagus fruits showed a similar picture to that of unripe red paprika fruits (Deli et al., 1996a). Unripe asparagus fruits contained the carotenoids typical of photosynthetic tissue (Table 1). The main carotenoids were lutein, β-carotene, and neoxanthin. As the fruit began to ripen, the chlorophyll content decreased, and lutein, β-carotene, and neoxanthin contents also decreased, while capsanthin and zeaxanthin contents increased. In contrast to ripe red paprika, in the ripe fruits of *A. officinalis*, zeaxanthin could be found as the main carotenoid and not capsanthin. We could also detect lutein in larger amounts, while ripe red paprika did not contain it. Furthermore, in the red paprika, we could always detect α-cryptoxanthin and α-carotene in small amounts, but we could not detect them in either the unripe or ripe asparagus fruits. Cucurbitaxanthin A with a 3,6-epoxy end group, which occurred in ~6% in red paprika, could not be detected either, similarly to other 3,6-epoxy or 3,5,6-trihydroxy carotenoids.

In earlier works (Deli et al., 1992, 1996a), we have depicted the biosynthesis of capsanthin and capsorubin from antheraxanthin in paprika. It can be seen from this scheme that four different biosynthetic routes exist for antheraxanthin. Out of these, the pinacol rearrangement into capsanthin is the most important. Furthermore, antheraxanthin may undergo epoxidation, epoxide ring opening, and endo epoxide rearrangement resulting in violaxanthin, 5,6-diepikarpoxanthin, and the carotenoid with a 3,6-epoxy end group, respectively. Since that time, we have isolated some carotenoids containing 3,5,6-trihydroxy β-end groups (6-epikarpoxanthin, 5,6-diepikarpoxanthin, 5,6-diepilatoxanthin, 5,6-diepicapsokarpoxanthin; Deli et al., 1998) and 3,6-epoxy β-end groups (cucurbitaxanthin A and B, cycloviolaxanthin, capsanthin 3,6-epoxide, cucurbitachrome epimers; Deli et al., 1996b) from red paprika.

In the asparagus fruits, we could not detect any 3,5,6-trihydroxy carotenoids or carotenoids with the 3,6-epoxy end group. If 3,5,6-trihydroxy carotenoids (e.g., 6-epikarpoxanthin, 5,6-diepikarpoxanthin, 5,6-diepilatoxanthin, 5,6-diepicapsokarpoxanthin) which might be expected on biosynthetic grounds are formed in the fruits of *A. falcatus*, they are present in quantities below the limits of detection. Our new results show that in the asparagus fruits the epoxide ring opening and endo epoxide rearrangement are not operative. The plausible biosynthetic transformations of antheraxanthin are summarized in Figure 3.

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