

Isolation and Identification of the Carotenoid Capsolutein from *Capsicum annuum* as Cucurbitaxanthin A

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Capsolutein, a carotenoid pigment mentioned to be present in *Capsicum annuum* but with an unknown structure, has been isolated. On the basis of spectroscopic (UV–visible, IR, and MS) and chromatographic (TLC and HPLC) behavior, as well as chemical derivatization, it has been identified to be identical with cucurbitaxanthin A (3',6'-epoxy-5',6'-dihydro- β,β -carotene-3,5'-diol), a peculiar carotenoid containing an 3,6-oxabicycloheptane end group. Hereafter the name cucurbitaxanthin A will be adopted for referring to capsolutein.

Keywords: *Capsicum annuum*; red pepper; carotenoid; capsolutein; cucurbitaxanthin A

INTRODUCTION

The carotenoid biosynthetic pathway in *Capsicum annuum* is complex (Curl, 1962; Davies et al., 1970), and in the past few years the number of pigments involved has been increased (Deli et al., 1992, 1994, 1995, 1996a). The novel carotenoids found in this genus are worthy of special attention, in particular those with the peculiar 3,6-oxabicycloheptane end group, such as cucurbitaxanthin A (3',6'-epoxy-5',6'-dihydro- β,β -carotene-3,5'-diol; **6**), cucurbitaxanthin B (5,6:3',6'-diepoxy-5,6,5',6'-tetrahydro- β,β -carotene-3,5'-diol; **7**), and cycloviolaxanthin (3,6:3',6'-diepoxy-5,6,5',6'-tetrahydro- β,β -carotene-3,5'-diol; **8**) (Parkes et al., 1986; Deli et al., 1991).

The unusual functional group 3,6-oxabicycloheptane was described for the first time when isolating three new carotenoids—eutreptiellanone ((3*S*,5*R*,6*S*)-3,6-epoxy-3',4',7',8'-tetrahydro-5,6-dihydro- β,β -caroten-4-one), β -cryptoeutreptiellanone ((3*S*,5*R*,6*S*, 3'*R*)-3'-hydroxy-3,6-epoxy-7',8'-didehydro-5,6-dihydro- β,β -caroten-4-one) and α -cryptoeutreptiellanone ((3*S*,5*R*,6*S*,3'*R*,6'*R*)-3'-hydroxy-3,6-epoxy-5,6-dihydro- β,ϵ -caroten-4-one)—from the marine alga *Eutreptilla gymnastica* (Fiksdahl et al., 1984; Bjørnland et al., 1986).

An additional two new carotenoids, cucurbitaxanthin A and cucurbitaxanthin B, containing this end group, were isolated from *Cucurbita maxima*, and their structures were elucidated (Matsuno et al., 1986). This was the first time that a pigment containing this functional group was described in higher plants. Later, Parkes et al. (1986) isolated several new pigments from *Capsicum annuum* with the 3,6-oxabicycloheptane end group, and determined their structure. Two of those pigments coincide with cucurbitaxanthin A and cucurbitaxanthin B. A third new pigment, cycloviolaxanthin, having two 3,6-oxabicycloheptane end groups, was discovered by Deli et al. (1991). Since then, there have been numerous studies reporting these pigments and other new ones, above all in Hungarian varieties of paprika (Deli et al., 1992, 1995, 1996a,b; Deli and Tóth, 1997).

In previous papers (Mínguez-Mosquera and Hornero-Méndez 1993, 1994a,b) we have reported, along with 14 other pigments in *C. annuum*, the presence of a pigment having the characteristics of the so-called capsolutein, although we did not state its complete structure, and only speculated on it from its chemical and UV–vis spectral properties. The pigment has now been isolated, identified, and its structure assigned. Some authors, such as Almela et al. (1990, 1991), reported the presence of this pigment in a wide range of crossed varieties but did not identify it. The data found in the literature indicate its identification as capsolutein, a pigment first described by Curl (1962), who established its nature as a diol xanthophyll with spectral and chromatographic characteristics similar to those of lutein (β,ϵ -carotene-3,3'-diol), so that it was named capsolutein (*Capsicum* lutein). The same author proposed the structure β,κ -caroten-3,3'-diol, and suggested that this should be half capsanthin (3,3'-dihydroxy- β,κ -caroten-6'-one; **3**) and half lutein. The same author speculated that its biosynthesis in the *Capsicum* pathway could be from capsanthin by reduction of the carbonyl to a $-\text{CH}_2-$. This carotenoid was reported as "Carotenoid of unknown structure" in the monograph *Carotenoids* (Straub, 1971). The aim of the present work is to confirm the structure of capsolutein and its correspondence to that of cucurbitaxanthin A shown by other authors.

EXPERIMENTAL PROCEDURES

Materials. Fruits of *Capsicum annuum* cv *Bola*, grown in the "La Vera" region (Cáceres, Spain), were used in their fully ripe stage.

Extraction and Isolation. The carotenoids from *C. annuum* fruits (100 g) were extracted with 100 mL of acetone, using a homogenizer (Polytron homogenizer Ultra-Turrax model T-25). Extraction was repeated until no further color was observed in the extracts. All fractions were pooled in a separating funnel and treated with 100 mL of diethyl ether, shaken, and left to settle. Enough NaCl solution (10% w/v) was added to separate the phases and in doing so transfer the pigments to the ether phase. This solution was treated several times with anhydrous Na_2SO_4 solution (2% w/v) to remove all the water. The ether phase, containing the pigments in different states of esterification with fatty acids, was saponified

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with 100 mL of KOH–MeOH (10% w/v), and left for 3 h with periodic shaking. After that pigments were transferred to diethyl ether by adding distilled water and the organic phase was washed to neutrality with more distilled water. Finally, the aqueous phase was removed and the organic phase was filtered through a bed of anhydrous Na₂SO₄ and evaporated to dryness in the rotary evaporator at a temperature lower than 35 °C. The pigments were dissolved with acetone to a volume of 25 mL and kept refrigerated at –20 °C until their analysis.

Standards. Lutein was isolated from a saponified extract of mint (*Mentha arvensis*, L.), by TLC (Mínguez-Mosquera and Hornero-Méndez, 1993). Zeaxanthin was obtained by TLC from fully ripe *Capsicum annuum* fruits (Mínguez-Mosquera and Hornero-Méndez, 1993). Isolation of authentic cucurbitaxanthin from *Cucurbita maxima* was achieved by following the method of Matsuno et al. (1986). For that purpose carotenoid pigments from pumpkin were extracted following the same procedure as for *C. annuum*. Preparative TLC on silica gel 60GF was used for the isolation as described by Matsuno et al. (1986), which uses the mixture benzene–EtOAc (3:1) as developer.

Isolation of Capsolutein from *C. annuum*. Capsolutein was isolated from a carotenoid extract from fully ripe *C. annuum* fruit by TLC on silica gel 60GF with petroleum ether (65–95 °C)–acetone–diethylamine (10:4:1) as developer. An orange band at *R_f* 0.47 was scraped off and eluted with acetone. Eventually, capsolutein was also isolated by semipreparative HPLC with a retention time of 9.10 min.

Spectroscopy. UV–visible spectra were obtained with a computerized PhotoDiode Array Spectrophotometer model HP 8452A (Hewlett-Packard) and recorded in Et₂O and benzene. IR spectra were recorded in KBr disks with an FST-7 Fourier transformed infrared spectrophotometer (Bio-Rad). Electron impact mass spectra were obtained with a VG model Quattro instrument with a direct insertion probe system at 70 eV ionizing voltage and ion source temperature 230–240 °C.

Purification of all carotenoids were carried out just before mass spectrometry by using a neutral alumina grade III minicolumn (Pasteur pipet).

TLC. Thin-layer chromatography, analytical and preparative, was carried out on silica gel GF60 plates in a presaturated chamber. The solvent system was the mixture petroleum ether (65–95 °C)–acetone–diethylamine (10:4:1).

HPLC. High-performance liquid chromatography was carried out using a Waters 600E pump coupled with a photodiode array detector (PDA) Waters model 996, and controlled by the Millennium 2010 computerized system. Detection was performed at 450 nm and spectra were recorded every 2 s in the range 350–600 nm with a resolution of 1 nm. For analytical purposes, the column used was a Spherisorb ODS 2 (25 × 0.46 cm) with 5 μm particle size, and for semipreparative scale, the column was Spherisorb ODS 2 (25 × 1 cm) with 5 μm particle size. Both columns were protected with a precolumn (1 × 0.46 cm) filled with the same material. The chromatographic method was the same as described by Mínguez-Mosquera and Hornero-Méndez (1993), and consist of a binary solvent gradient acetone–water at a flow rate of 1.5 and 6 mL/min for analytical and semipreparative chromatography, respectively. Samples were precleaned, either by centrifugation at 13 000 rpm or by filtration through a 0.45 μm Nylon membrane, prior to injection.

Pigment Identification. These have been described in detail in a previous publication (Mínguez-Mosquera and Hornero-Méndez, 1993), and consist of separation of pigment by TLC and cochromatography with purified pigments; observation of the pigment color on TLC plates under white, UV_{254nm} and UV_{360nm} lights with a Desaga UV–vis lamp; recording of UV–visible spectra in different solvents and comparison with the values reported in the literature (Foppen, 1971; Davies, 1976, 1988; Britton, 1991, 1995); as well as chemical derivatization microscale tests for the examination of 5,6-epoxide groups investigated by addition of 2% HCl in EtOH, acetylation with Ac₂O/Py to test for hydroxyl groups and reduction

with NaBH₄ in EtOH to test for carbonyl groups. Carbonyl and hydroxyl groups were also investigated by FT-IR spectroscopy.

Capsolutein was subjected to reduction with LiAlH₄. The pigment was dissolved in EtOH, and solid LiAlH₄ (few milligrams) was added. The reaction was monitored by TLC and HPLC.

Cucurbitaxanthin A (Also for capsolutein): *R_f* on TLC 0.47; *R_t* on HPLC 9.10 min; both pigments, cucurbitaxanthin and capsolutein, showed coelution; UV–vis λ_{max} nm 423, 445, 473 (Et₂O), 434, 458, 486 (benzene), nonhypsochromic shift upon treatment with HCl in EtOH; EI-MS (probe) 70 eV, *m/z* (rel int.) 584 [M]⁺ (100), 566 [M – 18]⁺ (10), 492 [M – 92]⁺ (5), 463 [M – 121]⁺ (10), 438 (5), 347 (18), 228 (25); IR ν_{max} cm⁻¹ 3370 (*m*) (broad), 2920 (*s*), 2856 (*m*), 1453 (*w*), 1367 (*w*), 1350 (*w*), 1296 (*w*), 1213 (*w*), 1091 (*m*), 1028 (*m*), 866 (*w*), 820 (*w*).

Zeaxanthin: *R_f* on TLC 0.42; *R_t* on HPLC 9.4 min; UV–vis λ_{max} nm 422, 448, 472 (Et₂O), 436, 462, 488 (benzene); EI-MS (probe) 70 eV, *m/z* (rel int.) 568 [M]⁺ (100), 550 [M – 18]⁺ (20), 532 [M – 36]⁺ (10), 476 [M – 92]⁺ (45), 462 [M – 106]⁺ (6).

Lutein: *R_f* on TLC 0.40; *R_t* on HPLC 9.3 min, UV–vis λ_{max} nm 420, 442, 472 (Et₂O), 430, 456, 486 (benzene); EI-MS (probe) 70 eV, *m/z* (rel int.) 568 [M]⁺ (60), 550 [M – 18]⁺ (100), 532 [M – 36]⁺ (50), 476 [M – 92]⁺ (10), 462 [M – 106]⁺ (5).

RESULTS AND DISCUSSION

Capsolutein was isolated initially by TLC on silica gel GF60 according to the method described previously (Mínguez-Mosquera and Hornero-Méndez, 1993). It presented an *R_f* of 0.47. Next, preparative HPLC was used to monitor in situ the purity of the isolated fractions. The pigment shows an orange color under white light. The concentration levels of this pigment in ripe fruits of red pepper are around 80 mg kg⁻¹ of fresh fruit, similar to the levels of zeaxanthin (β,β-carotene-3,3'-diol) and β-carotene (β,β-carotene), so that it can be considered as a major pigment (Mínguez-Mosquera and Hornero-Méndez, 1994a). The spectroscopic characteristics in visible light, 425, 445, 473 nm (Et₂O), indicate nine conjugated double bonds in the central chromophore and one double bond conjugated with the chain in a terminal ring—that is, a β-type ring (Britton, 1995). Such characteristics might suggest lutein, but this was ruled out by comparison with lutein standard in both TLC and HPLC. At the same time, the pigment showed a molecular ion with *m/z* 584, which does not correspond with that of lutein (*m/z* 568), and the fragment ion [M – 18 – 18]⁺ (*m/z* 532) for lutein was also not shown. The *m/z* value for the molecular ion of capsolutein is consistent with a formula such as C₄₀H₅₆O₃, which corresponds with the formula of cucurbitaxanthin A. The presence of the [M – 18]⁺ (*m/z* 566) ion in the mass spectrum indicates the presence of at least one hydroxyl group, which was confirmed by IR (absorption at 3370 cm⁻¹). Acetylation and observation of the resulting products by TLC showed the presence of two hydroxyl groups, one of which must be at a position not facilitating a second dehydration and thus the presence of the fragment ion [M – 18 – 18]⁺ in the mass spectrum. This again coincides with what is observed in cucurbitaxanthin A, in which there is a secondary –OH on a β-type ring and a tertiary –OH in the other end group.

The pigment developed on TLC was subjected to the classic test for detection of 5,6- and 5,8-epoxide groups by acid treatment with HCl. It gave the characteristic blue color, which indicated the presence of such groups. However, when the test was carried out with spectro-

photometric control in ethanol, no hypsochromic displacement was observed. Nor were $[M - 80]^+$ or $[M - 106]^+$ fragments, characteristic of epoxides, seen in the mass spectrum. This is in accord with the possible presence of the 3,6-epoxide group, a functional group that does not show the characteristic hypsochromic displacement when treated with acid (Eugster, 1995), and which is present in cucurbitaxanthin A.

Authentic cucurbitaxanthin A isolated from *Cucurbita maxima* by TLC and preparative HPLC showed accord with everything observed up to now for capsolutein, besides coeluting in TLC and HPLC systems. As a final test, capsolutein was subjected to reduction with LiAlH_4 , yielding zeaxanthin. The reaction was monitored by HPLC. This same product was obtained by Matsuno et al. (1986) after subjecting cucurbitaxanthin A to the same reaction.

Thus we must conclude that capsolutein and cucurbitaxanthin A are synonyms for the same pigment in *C. annuum*. We will use the name cucurbitaxanthin A hereafter. Nevertheless, chronologically, the name capsolutein was more appropriate, since it was that given by Curl (1962), although he was not to assign its structure, as at that time only the UV-visible spectrum and phase separation study were available.

The biosynthesis of this pigment seems to be complex and situated in the last steps of the biosynthetic pathway (Figure 1). This notion is supported by previous research (Mínguez-Mosquera and Hornero-Méndez, 1994b) showing that the chronological appearance of the pigment during the ripening of pepper fruits is observed in the last stages, reaching a maximum concentration when the fruit is overripe. At the same time, it has been observed that when the fruits are subjected to mild heat treatment before their processing as paprika, pigment synthesis continues (Mínguez-Mosquera and Hornero-Méndez, 1994a). This is not de novo, but takes place at the expense of already existing precursor pigments [mainly β,β -carotene, β -cryptoxanthin (β,β -caroten-3-ol), zeaxanthin, and violaxanthin (5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β,β -carotene-3,3'-diol; **2**)], leading to an increase in the concentration of certain pigments such as capsanthin, capsorubin (3,3'-dihydroxy- κ,κ -carotene-6,6'-dione; **5**) and capsolutein among others. At the same time, when the carotenoid composition of a pepper having a final yellow coloration is analyzed (Matus et al., 1991), capsolutein is always absent, as are the normal pigments of pepper that contain acylcyclopentanol groups. In this case, the conversion of the 3-hydroxy-5,6-epoxide group (formed on the β -ring) to the acylcyclopentanol group by pinacollic rearrangement is blocked, so that pigments such as antheraxanthin (5,6-epoxy-5,6-dihydro- β,β -carotene-3,3'-diol; **1**), violaxanthin, and β -cryptoxanthin-5,6-epoxide (5,6-epoxy-5,6-dihydro- β,β -caroten-3-ol) accumulate. These are precursors of the red carotenoids—capsanthin, capsorubin, and capsanthin-5,6-epoxide—which are biosynthesized by pinacollic rearrangement of 3-hydroxy 5,6-epoxide end groups in normal red pepper fruits (Scheme 1 and Figure 1). This leads us to assume with confidence that capsolutein (cucurbitaxanthin A) is also biosynthesized from a 3-hydroxy 5,6-epoxide group by another type of rearrangement, with its immediate precursor seeming to be antheraxanthin. A possible mechanism might be by the rearrangement of the 3-hydroxy-5,6-epoxide end group (Scheme 2).

Cucurbitaxanthin B would be formed by the same mechanism (Figure 1) starting from violaxanthin, by rearrangement of a single 3-hydroxy 5,6-epoxide group,

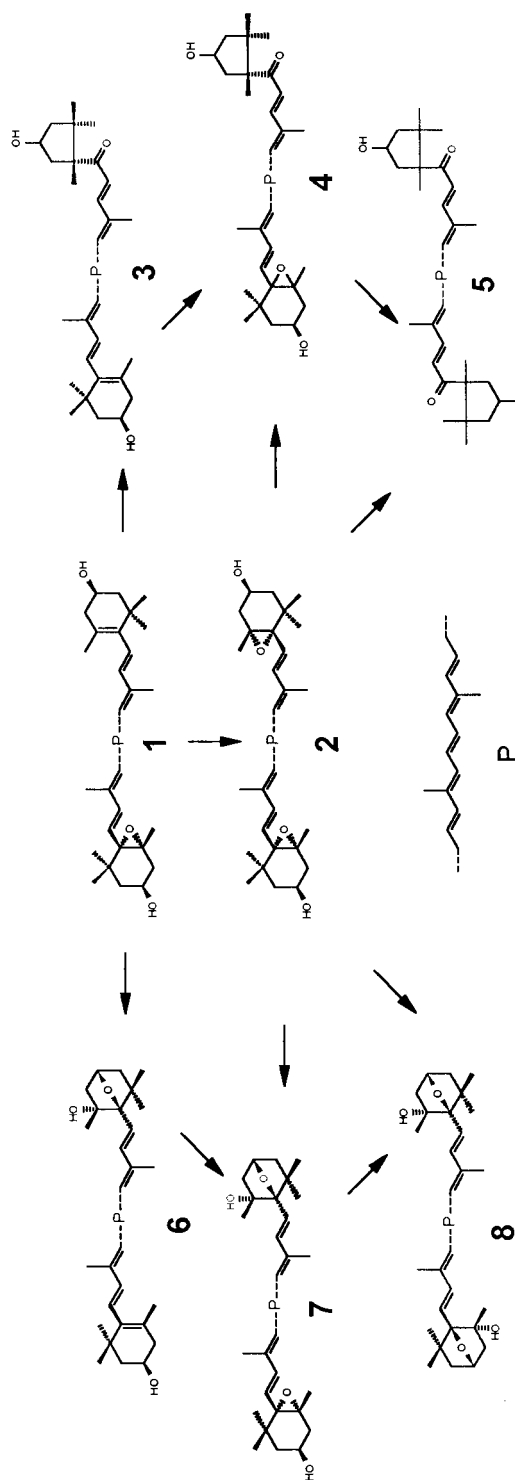
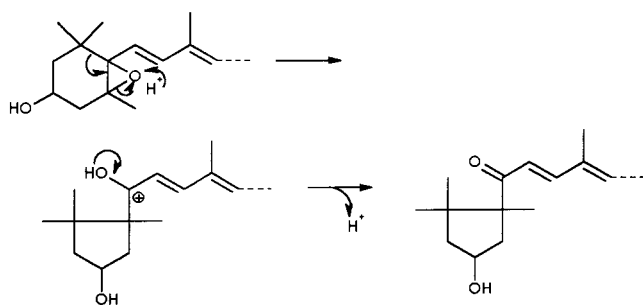
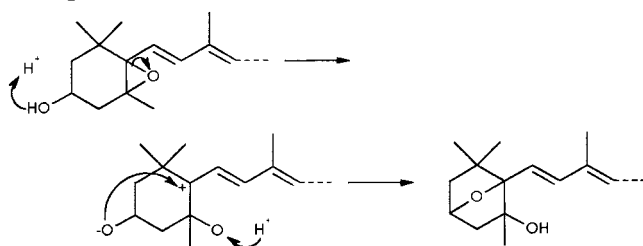


Figure 1. Biosynthetic pathway of acylcyclopentanol end group containing pigments and possible pathway for 5-hydroxy-3,6-oxabicycloheptane end group containing pigments in *Capsicum annuum*: (1) antheraxanthin, (2) violaxanthin, (3) capsanthin, (4) capsorubin, (5) capsanthin 5,6-epoxide, (6) capsolutein, (7) cucurbitaxanthin A, and (8) cyclovioaxanthin.

Scheme 1. Pinacolic Rearrangement from 3-Hydroxy 5,6-Epoxy to Acylcyclopentanol End Group



Scheme 2. Possible Rearrangement from 3-Hydroxy 5,6-Epoxy to 5-Hydroxy-3,6-oxabicycloheptane End Group



and cycloviolaxanthin (which contains two 3,6-oxabicycloheptane terminal groups) by the rearrangement of both groups. The latter pigment has been described in black varieties of hungarian pepper (Deli et al., 1991, 1992), but has not been detected in our red varieties. Cycloviolaxanthin could also be formed in the same way from cucurbitaxanthin B. Similarly, by this type of rearrangement capsanthin 5,6-epoxide (5,6-epoxy-3,3'-dihydroxy-5,6-dihydro- β,κ -caroten-6'-one; **4**) would give rise to capsanthin 3,6-epoxide (3,6-epoxy-5,3'-dihydroxy-5,6-dihydro- β,κ -caroten-6'-one), also found in black varieties (Deli et al., 1991). The latter pigment could also be formed from cucurbitaxanthin B by the pinacolic rearrangement of the 3-hydroxy 5,6-epoxide group.

To sum up, the pigment family comprising cucurbitaxanthin A, cucurbitaxanthin B, and cycloviolaxanthin is equivalent to the group capsanthin, capsanthin 5,6-epoxide, and capsorubin, respectively, but generated by a nonpinacolic rearrangement of the 3-hydroxy 5,6-epoxide group that is still to be investigated.

LITERATURE CITED

- Almela, L.; López-Roca, J.; Candela, M. E.; Alcázar, M. D. Separation and determination of individual carotenoides in a *Capsicum* cultivar by normal phase high-performance liquid chromatography. *J. Chromatogr.* **1990**, *502*, 95–106.
- Almela, L.; López-Roca, J.; Candela, M. E.; Alcázar, M. D. Carotenoid composition of new cultivars of red pepper for paprika. *J. Agric. Food Chem.* **1991**, *39*, 1606–1609.
- Björnland, T.; Borch, G.; Liaaen-Jensen, S. Additional oxabicyclo[2.2.1] heptane carotenoids from *Eutreptiella gymnastica*. *Phytochemistry* **1986**, *25*, 201–205.
- Britton, G. Carotenoids. In *Methods in Plant Biochemistry*; Academic Press Limited: San Diego, CA, 1991; Vol. 7, Chapter 13.
- Britton, G. UV/Visible spectroscopy. In *Carotenoids Vol 1B: Spectroscopy*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Eds.; Birkhäuser: Basel, 1995; Chapter 2.
- Curl, A. L. The carotenoids of red bell peppers. *J. Agric. Food Chem.* **1962**, *10*, 504–509.
- Davies, B. H. Carotenoids. In *Chemistry and Biochemistry of plant pigments*; Goodwin, T. W., Ed.; Academic Press: London, 1976; Vol. 2, Chapter 19.
- Davies, B. H.; Köst, H. P. Carotenoids. In *Handbook of Chromatography*; Köst, H. P., Ed.; CRC Press: Boca Raton, 1988; pp 93–117.
- Davies, B. H.; Matthews, S.; Kirk, J. T. O. The Nature and biosynthesis of the carotenoids of different colour varieties of *Capsicum annuum*. *Phytochemistry* **1970**, *9*, 797–805.
- Deli, J.; Molnár, P.; Tóth, G.; Baumeler, A.; Eugster, C. H. Cycloviolaxanthin (=3*S*,5*R*,6*R*,3'*S*,5'*R*,6'*R*)-3,6,3',6'-Diepoxo-5,6,5',6'-tetrahydro- β,β -carotene-5,5'-diol), a novel carotenoid from red paprika. *Helv. Chim. Acta* **1991**, *74*, 819–824.
- Deli, J.; Matus, Z.; Szabolcs, J. Carotenoid composition in the fruits of black paprika (*Capsicum annuum* variety *longum nigrum*) during ripening. *J. Agric. Food Chem.* **1992**, *40*, 2072–2076.
- 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.
- 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 -carotene-3',6'-dione) from paprika (*Capsicum annuum*). *Chimia* **1995**, *49*, 69–71.
- Deli, J.; Molnár, P.; Matus, Z.; Tóth, G.; Steck, A. Reisolation of carotenoid 3,6-epoxides from red paprika (*Capsicum annuum*). *Helv. Chim. Acta* **1996a**, *79*, 1435–1443.
- Deli, J.; Matus, Z.; Tóth, G. Carotenoid composition in the fruits of *Capsicum annuum* cv. Szentesi Kosszarvú during ripening. *J. Agric. Food Chem.* **1996b**, *44*, 711–716.
- Deli, J.; Tóth, G. Carotenoid composition of the fruits of *Capsicum annuum* cv. Bovet 4 during ripening. *Z. Lebensm.-Unters.-Forsch. A* **1997**, *205*, 388–391.
- Eugster, C. H. Chemical derivatization: Microscale tests for the presence of common functional groups in carotenoids. In *Carotenoids*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1A (Isolation and Analysis), Chapter 4.
- Fiksdahl, A.; Björnland, T.; Liaaen-Jensen, S. Algal carotenoids with novel end groups. *Phytochemistry* **1984**, *23*, 649–655.
- Foppen, F. H. Tables for identification of carotenoid pigments. *Chromatogr. Rev.* **1971**, *14*, 133–298.
- Matsuno, T.; Tani, Y.; Maoka, T.; Matsuo, K.; Komori, T. Isolation and structural elucidation of cucurbitaxanthin A and B from pumpkin *Cucurbita maxima*. *Phytochemistry* **1986**, *25*, 2837–2840.
- 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.
- Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reversed-phase HPLC. *J. Agric. Food Chem.* **1993**, *41*, 1616–1620.
- Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Formation and transformation of pigments during the fruit ripening of *Capsicum annuum* cv. Bola and Agridulce. *J. Agric. Food Chem.* **1994a**, *42*, 38–44.
- Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Comparative study of the effect of paprika processing on the carotenoids in peppers (*Capsicum annuum*) of the Bola and Agridulce varieties. *J. Agric. Food Chem.* **1994b**, *42*, 1555–1560.
- 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*, 2535–2538.
- Straub, O. List of natural carotenoids. In *Carotenoids*; Isler, O., Ed.; Birkhäuser: Basel, 1971; Chapter XII.

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