Isolation of Carotenoids with 3,5,6-Trihydroxy-5,6-dihydro-β-end Groups from Red Paprika (*Capsicum annuum*)

by **József Deli**[a]), **Péter Molnár**[a]), **Zoltán Matus**[a]), **Gyula Tóth**[a])*, **Andrea Steck**[b]), and **Hanspeter Pfander**[b])*

[a]) Department of Medical Chemistry, University Medical School of Pécs, P.O. Box 99, H-7601 Pécs
[b]) Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern

6-Epikarpoxanthin ((all-*E*,3*S*,5*R*,6*S*,3′*R*)-5,6-dihydro-β,β-carotene-3,5,6,3′-tetrol, **5**), 5,6-diepikarpoxanthin ((all-*E*,3*S*,5*S*,6*S*,3′*R*)-5,6-dihydro-β,β-carotene-3,5,6,3′-tetrol, **13**), 5,6-diepilatoxanthin ((all-*E*,3*S*,5*S*,6*S*,3′*S*,5′*R*,6′*S*)-5′,6′-epoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,6,3′-tetrol, **14**), and 5,6-diepicapsokarpoxanthin ((all-*E*,3*S*,5*S*,6*S*,3′*S*,5′*R*)-5,6-dihydro-3,5,6,3′-tetrahydroxy-β,κ-caroten-6′-one, **15**) were isolated from red spice paprika (*Capsicum annuum* var. *longum*) and characterized by their UV/VIS, CD, ^{1}H- and ^{13}C-NMR, and mass spectra. Our investigations demonstrate that the configuration of the 3,5,6-trihydroxy-5,6-dihydro-β-end group may differ depending on the biological source.

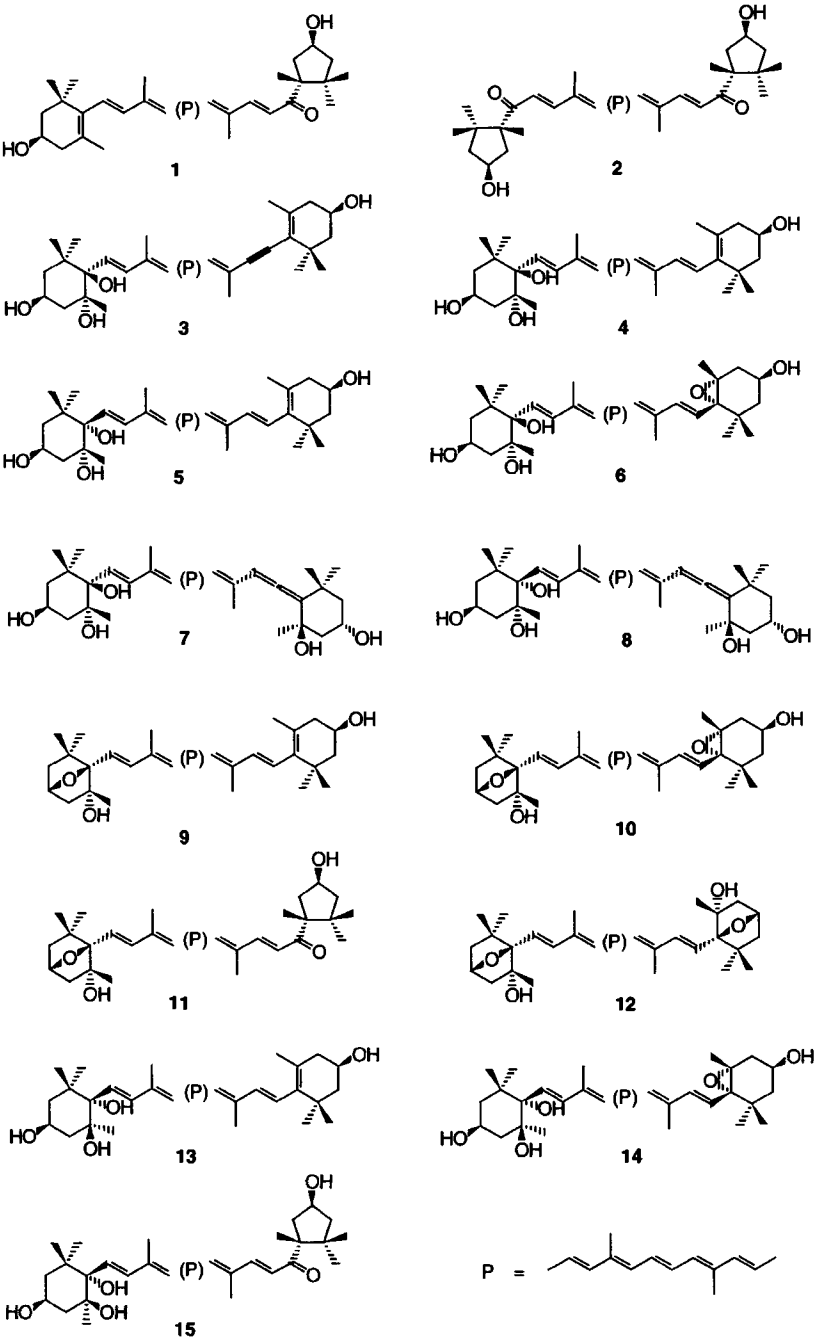
1. Introduction. – Different varieties of paprika (*Capsicum annuum*) have been investigated for a long time. It has been established that capsanthin (**1**) and capsorubin (**2**), both containing the five-membered ring κ-end group, are the most abundant carotenoids in these vegetables. Furthermore, many other carotenoids with interesting structures, especially those with the oxabicyclo-β-end group and the 3,5,6-trihydroxy-5,6-dihydro-β-end group, have been isolated [1][2].

Naturally occurring carotenoids with the 3,5,6-trihydroxy-5,6-dihydro-β-end group have been isolated from different sources. Heteroxanthin (**3**) was isolated from *Euglena gracilis* [3], karpoxanthin (**4**) from ripe hips of *Rosa pomifera* [4], karpoxanthin (**4**) and 6-epikarpoxanthin (**5**) from petals and pollen of *Lilium trigrinum* [5], latoxanthin (**6**) from petals of *Rosa foetida* [6], and neoflor (**7**) and 6-epineoflor (**8**) from petals of *Trollius europaeus* [7].

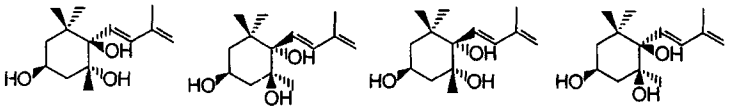
The structure elucidation of the 3,5,6-trihydroxy-5,6-dihydro-β-end group was performed by *Eugster* and co-workers [8][9]. Several ionone derivatives and carotenoids bearing this end group were prepared by partial synthesis, and the spectroscopic data for the 3,5,6-trihydroxy-5,6-dihydro-β-end group with the (3*S*,5*R*,6*R*) (**A**), (3*S*,5*S*,6*S*) (**B**), (3*S*,5*R*,6*S*) (**C**), and (3*S*,5*S*,6*R*) (**D**) configuration have been established.

Based on these results, the configuration of the 3,5,6-trihydroxy-5,6-dihydro-β-end group was established as (3*S*,5*R*,6*R*) (**A**) in karpoxanthin ((3*S*,5*R*,6*R*)-**4**) originating from ripe hips of *Rosa pomifera* [4], in latoxanthin ((3*S*,5*R*,6*R*)-**6**) originating from *Rosa foetida* [6], and in neoflor ((3*S*,5*R*,6*R*)-**7**) originating from *Trollius europaeus* [7], and as (3*S*,5*R*,6*S*) (**C**) in 6-epikarpoxanthin ((3*S*,5*R*,6*S*)-**5**) originating from ripe hips of *Rosa pomifera* [4] and in 6-epineoflor ((3*S*,5*R*,6*S*)-**8**) originating from *Trollius europaeus* [7]. In contrast, it is of great interest that for karpoxanthin, isolated from red paprika, the (3*S*,5*S*,6*S*)-configuration (**B**), *i.e.*, (3*S*,5*S*,6*S*,3′*S*)-**4**, was established.

During our investigations of different species of paprika (*Capsicum annuum*), some novel carotenoids with the 7-oxabicyclo[2.2.1]heptyl-end group (3,6-epoxycyclohexyl)



such as cucurbitaxanthins A (**9**) and B (**10**), capsanthin-3,6-epoxide (**11**), and cycloviolaxanthin (**12**) have been isolated and characterized [10]. In the possible biosynthetic route for the formation of the 3,6-epoxy-end group from a 5,6-epoxy ring, the 3,5,6-tri-



A (3*S*,5*R*,6*R*) **B** (3*S*,5*S*,6*S*) **C** (3*S*,5*R*,6*S*) **D** (3*S*,5*S*,6*R*)

hydroxy compounds may be intermediates. As a continuation of our work on paprika carotenoids, we report in the present paper the structure elucidation of the four carotenoids **5** and **13**–**15** bearing the 3,5,6-trihydroxy-5,6-dihydro-β-end group which were isolated from red spice paprika (*Capsicum annuum* var. *longum*).

2. Results and Discussion. – The fresh ripe paprika pods were first extracted with MeOH and, after saponification, the residue was distributed between hexane and MeOH/H_2O 9:1. The hypophasic pigments were precipitated from benzene/hexane. After repeated column chromatography (see *Exper. Part*) and crystallization, the carotenoids **5** and **13**–**15** were isolated.

Spectroscopic Characterization. In the UV/VIS spectra, the maxima for **5** and **13** (434, 457, and 458, 487 nm, resp., in benzene) as well as for **14** (427, 453, and 483 nm in benzene), and also the fine structure were in accordance with the data previously reported for karpoxanthin (**4**) and latoxanthin (**6**) [4][6]. In addition, also the product from the furanoid rearrangement of **14** exhibited the expected absorption maxima (407, 431, and 460 nm in benzene). The UV/VIS spectrum of **15** (480 and 505 (shoulder) nm in benzene) was in agreement with a decaene chromophore including a conjugated C=O group. Reduction of **15** with $NaBH_4$ gave a mixture (*ca.* 1:1) of the corresponding stereoisomeric alcohols. The UV/VIS spectrum of this mixture exhibited, as expected, increased fine structure and a hypsochromic shift (426, 451, 481 nm in benzene).

In each case, the mass spectra showed the corresponding molecular-ion peaks. In addition to the signals typical for hydroxy carotenoids ($[M - H_2O]^+$, $[M -$ toluene$]^+$), strong peaks at *m/z* 221 and 181, characterizing the 3,5,6-trihydroxy-end group, were observed.

Eugster and co-workers investigated the ring-opening reactions of 3-hydroxy-5,6-epoxy carotenoids by hydrolysis and determined the configuration of the 3,5,6-trihydroxy products [8]. By synthesis or partial synthesis of compounds with the stereoisomeric 3,5,6-trihydroxy-end groups **A**–**D** from starting products with well-known absolute configurations and by comparison of the ^{1}H-NMR data [8], a set of diagnostically relevant ^{1}H chemical shifts were obtained for each stereoisomer. The shift patterns of the four stereoisomeric end groups, including δ values for H–C(3), H–C(7), Me(16), Me(17), and Me(18) exhibited sufficient differences to allow determination of the configuration of the 3,5,6-trihydroxy-end group. Later NMR investigations on 3,5,6-trihydroxy carotenoids [4][8][11] confirmed these results.

The comparison of the ^{1}H-NMR data of the 3,5,6-trihydroxy-end groups of **13**–**15** with *Eugster*'s data [8] showed agreement with the end group **B** that has the (3*S*,5*S*,6*S*)-configuration. This was confirmed by further NMR investigations, including COSY, T ROESY, ^{13}C, DEPT-135, and inverse HMQC experiments (see *Exper. Part*). In contrast, the ^{1}H-NMR data for the trihydroxy-end group of **5** were in agreement with the end group **C** with the (3*S*,5*R*,6*S*)-configuration.

In addition it should be mentioned that the $\delta(^1H)$ assignments for Me(17) and Me(18) in the 3,5,6-trihydroxy-end group in [4][7][8] are reversed in our studies. COSY Spectra show intensive Me(16)/Me(17) cross-peaks and clearly confirm our line assignments.

Compounds **5** and **13** showed similar CD spectra with positive maxima at 241 and 248 nm, and negative maxima at 276 and 283 nm, respectively, which were in agreement with the CD spectrum of karpoxanthin (**4**) [4] (*Fig.*). Compound **14** exhibited a CD spectrum with positive maxima at 209, 245, and 305 nm, and negative maxima at 227, 268, and 283 nm, in agreement with the data for latoxanthin (**6**) [6]. In the CD spectrum of **15**, positive maxima at 208 and 286 nm, and negative maxima at 204 and 240 nm were observed.

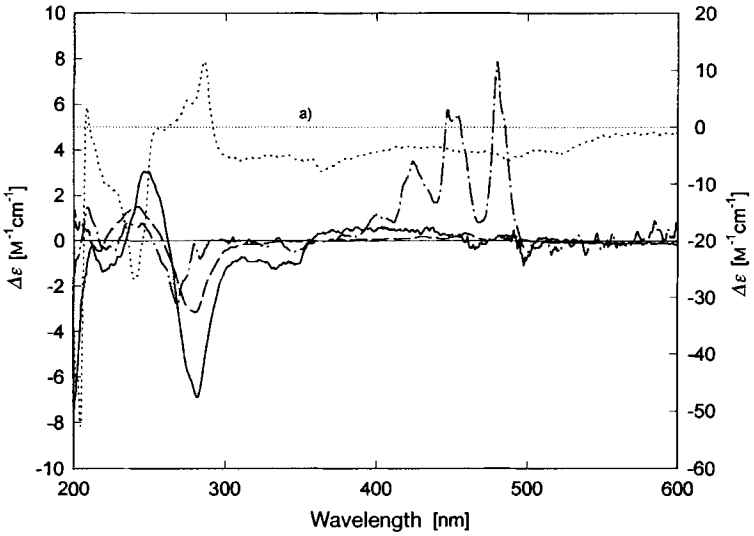


Figure. *CD Spectra of 5,6-diakarpoxanthin* (——, **13**), *6-epikarpoxanthin* (– – –, **5**), *5,6-diepilatoxanthin* (– · – · –, **14**), *5,6-diepicapsokarpoxanthin* (· · · · · · ·, **15**) *in EPA at* $-180°$. a) Null line for 5,6-dicapsokarpoxanthin (**15**).

Based on our spectroscopic data, the structures of the four carotenoids **5** and **13**–**15** were unequivocally assigned. Compound **5** was identical with 6-epikarpoxanthin (= (all-*E*,3*S*,5*R*,6*S*,3′*R*)-5,6-dihydro-β,β-carotene-3,5,6,3′-tetrol), previously isolated from the petals and pollen of *Lilium tigrinum* [5]. Carotenoid **13** was identified as (all-*E*,3*S*,5*S*,6*S*,3′*S*)-5,6-dihydro-β,β-carotene-3,5,6,3′-tetrol. Since it is a diastereoisomer of karpoxanthin **4** with inversed configuration at C(5) and C(6), we propose the trivial name 5,6-diepikarpoxanthin for **13**. Similarly, **14** was identified as (all-*E*,3*S*,5*S*,6*S*,3′*S*,5′*R*, 6′*S*)-5′,6′-epoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,6,3′-tetrol, and for this 5,6-diastereoisomer of latoxanthin **6** the name 5,6-diepilatoxanthin is proposed. The 3,5,6-trihydroxy carotenoid **15** contains as its second end group the κ-end group and represents a carotenoid with a hitherto unknown constitution. The compound was identified as (all-*E*,3*S*,5*S*,6*S*,3′*S*,5′*R*)-5,6-dihydro-3,5,6,3′-tetrahydroxy-β,κ-caroten-6′-one, and, by analogy to **13** and **14**, the name 5,6-diepicapsokarpoxanthin is proposed. Co-chromatography by HPLC of the isolated carotenoid **15** with semi-synthetic (all-

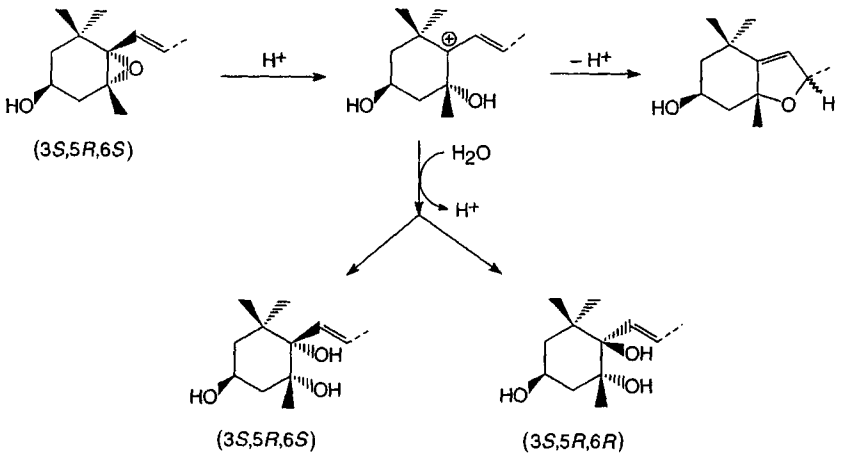
E,3*S*,5*R*,6*R*,3′*S*,5′*R*)- and (all-*E*,3*S*,5*R*,6*S*,3′*S*,5′*R*)-capsokarpoxanthin, prepared from '*anti*'-capsanthin-5,6-epoxide, and with (all-*E*,3*S*,5*S*,6*R*,3′*S*,5′*R*)-capsokarpoxanthin, obtained, from '*syn*'-capsanthin-5,6-epoxide [12], showed no identity, supporting our structure assignment.

Discussion. Our investigations demonstrate that the sense of chirality of karpoxanthin and latoxanthin differs depending on the natural sources. In paprika, the configuration of **13** and **14** was established as (3*S*,5*S*,6*S*), whereas in other organisms it is (3*S*,5*R*,6*R*). In addition, capsokarpoxanthin (**15**), which has been isolated for the first time from natural sources, exhibits the (3*S*,5*S*,6*S*)-configuration. In contrast, 6-epikarpoxanthin (**5**) possesses the (3*S*,5*R*,6*S*)-chirality independent of its natural source.

It can be assumed that in Nature the carotenoids with the 3,5,6-trihydroxy-end group are formed from the 3-hydroxy-5,6-epoxy-end group, the (3*S*,5*R*,6*S*)-3-hydroxy-5,6-epoxy-β-end group of violaxanthin being a possible precursor in all organisms. Therefore, it is highly probable that, in paprika, compared to other organisms, the 3,5,6-trihydroxy-end group is formed from the corresponding 3-hydroxy-5,6-epoxy-end group by a different mechanism.

It has been established [9] that the acid-catalyzed hydrolysis of the (3*S*,5*R*,6*S*)-3-hydroxy-5,6-epoxy-end group results in a mixture of compounds with the (3*S*,5*R*,6*R*)- and (3*S*,5*R*,6*S*)-3,5,6-trihydroxy-end groups, *i.e.*, with retention of configuration at C(5). This can be explained by the formation of a carbenium ion at C(6) (*Scheme 1*).

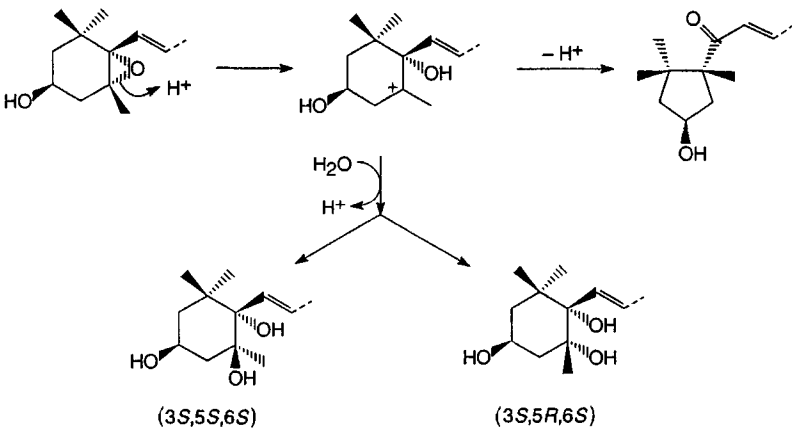
Scheme 1



On the other hand, the formation of a mixture of the (3*S*,5*S*,6*S*)- and (3*S*,5*R*,6*S*)-3,5,6-trihydroxy-end group from the (3*S*,5*R*,6*S*)-3-hydroxy-5,6-epoxy-end group can be explained by the formation of carbenium ion at C(5) (*Scheme 2*).

The formation of a carbenium ion at C(5) as intermediate has been proposed in the mechanism of the conversion of antheraxanthin and violaxanthin to the κ-rings of capsanthin (**1**) and capsorubin (**2**), the most important carotenoids in paprika. As shown in *Scheme 2* the mechanism involves a pinacol rearrangement.

Scheme 2



Recently, the capsanthin-capsorubin synthase (CCS), an enzyme catalyzing the conversion of 5,6-epoxy-end groups into κ-end groups, was isolated and characterized [13], and certain similarities with the *C. annuum* lycopene cyclase, the enzyme catalyzing the cyclization of lycopene, were observed [14]. The fact that CCS exhibited also lycopene cyclase activity is likely to be related to 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 [15]. On the basis of the above described reaction mechanism, 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 C(5). Therefore, during the enzyme-catalyzed hydrolysis of 5,6-epoxy carotenoids, the configuration at C(5) may change, and the configuration at C(6) remains unchanged. It should be noted that, until now, it has not been clarified whether the 3,5,6-trihydroxy carotenoids are intermediates or by-products of pinacol rearrangement. The resolution of this question demands further biochemical investigations.

In the plants (*Rosa foetida*, ripe hips of *Rosa pomifera*) that do not contain carotenoids with κ-end group, the ring opening of 5,6-epoxy carotenoids may be acid-catalyzed. During the acid-catalyzed hydrolysis of 5,6-epoxy carotenoids, the configuration at C(6) may change, and that at C(5) remains unchanged. The different biosynthetic routes may explain the differences between the configurations of carotenoids with 3,5,6-trihydroxy-β-end group isolated from different sources.

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Experimental Part

1. *General.* TLC: silica gel F_{254} (*Merck 5554*), benzene/AcOEt/MeOH 7:2:1. HPLC: *Gynkotek* pump model *300 B* with *Gynkotek* gradient former, detector: *Waters-991*, photodiode array. Colomn: 250 × 4.6 mm i.d.,

Chromsyl C_{18}, 6 μm, endcapped. Mobile phase: *A* 12% H_2O in MeOH, *B*: MeOH, *C*: acetone/MeOH 1:1. Gradient program: 0–2 min: 100% *A*; 2–10 min: to 80% *A*/20% *B*; 10–18 min: to 50% *A*/50% *B*; 18–25 min: to 100% *B*; 25–27 min: 100% *B*; 27–34: to 100% *C*; 34–41 min 100% *C* (linear steps). UV/VIS: *Beckman DU-65*. CD: *Jobin-Yvon Dichrograph CD-6*. Solvent: EPA (Et_2O/isopentane/EtOH/5:5:2). NMR: *Bruker DRX 400* (^{1}H: 400.14 MHz, ^{13}C: 100.61 MHz), solvent: $CDCl_3$, *T* 20°, δ in ppm (relative to the solvent signal), *J* in Hz. MS: *Varian MA-CH 7A*; *m/z* (rel. intensity in %).

2. *Isolation and Separation*. A detailed description of the general extraction procedure has been reported in [10]. The 450 mg hypophasic crystalline product originated from MeOH extract was submitted to CC: 16 column 6 × 30 cm, $CaCO_3$ (*Biogal*, Hungary), benzene. The picture after development: band *1*: 12 mm brick red (mixture containing **15**); band *2*: 15 mm violet (capsorubin, **5** and **14**); band *3*; 10 mm yellow (**13**); band *4*: 20 mm pink (mixture 5,6-epoxycapsanthin/3,6-epoxycapsanthin); band *5*: 40 mm red (capsanthin); band *6*: 6 mm yellow (violaxanthin and cucurbitaxanthin B); band *7*: 15 mm pale-yellow (cucurbitaxanthin A and zeaxanthin). After the processing, the bands *4*–*7* were crystallized from benzene by addition of hexane; yield: 16 mg of a mixture 3,6-epoxycapsanthin/5,6-epoxycapsanthin, 216 mg of capsanthin, 20 mg of a mixture violaxanthin/cucurbitaxanthin B, and 35 mg of a mixture of cucurbitaxanthin A/zeaxanthin, resp.

5,6-Diepikarpoxanthin (**13**): After the processing, the band *3* was crystallized (benzene/hexane) to give 24 mg of red crystals (m.p. 150°), with a purity of 97%.

5,6-Diepicapsokarpoxanthin (**15**): The zone *1* was subsequently submitted to a second CC: 6 column 6 × 30 cm, $CaCO_3$ (*Biogal*, Hungary), 4% acetone in benzene. The picture after development: 10 mm pale-yellow (unknown), 8 mm pink ((*Z*)-capsanthins), 20 mm pink ((*Z*)-capsanthins), 20 mm red (**15**). The zone containing **15** was submitted to a third CC: 3 column 6 × 30 cm, $CaCO_3$ (*Biogal*, Hungary), 6% acetone in hexane. Picture after development: 2 mm pale-yellow (unidentified), 8 mm ochre (unidentified), 12 mm red (**15**), 8 mm ochre (unidentified). After desorption, **15** was crystallized (benzene/acetone/hexane) to give 4.2 mg of red crystals, with a purity of 99%.

5,6-Diepilatoxanthin (**14**) *and 6-Epikarpoxantin* (**5**): The zone *2* was subsequently submitted to a second CC: 4 column 6 × 30 cm, $CaCO_3$ (*Biogal*, Hungary), 5% acetone in hexane. The picture after development: 60 mm pink (unidentified), 10 mm red (capsorubin, **5**, and **14**), 3 mm pale-yellow (capsochrome), 3 mm pink (unidentified), 5 mm pale-yellow (unidentified). The zone containing **5** and **14** was submitted to a third CC: 3 column 6 × 30 cm, MgO-*Celite* (1:1) 4% acetone in benzene. Picture after development: 5 mm red (capsorubin), 10 mm yellow (**5** and **14**). The zone containing **5** and **14** submitted to a fourth CC: 2 column 6 × 30 cm, $CaCO_3$ (*Biogal*, Hungary), 3–6% acetone in hexane. Picture after development: 5 mm pale-yellow (**5**), 1 mm ochre, 10 mm yellow (**14**). After desorption, **14** was crystallized (benzene/hexane) to give 0.8 mg of orange crystals, with a purity of 96%. The 6-epikarpoxanthin (**5**) was crystallized (benzene/hexane) to give 0.8 mg of red crystals, with a purity of 95%.

3. *6-Epikarpoxanthin* (*(3S,5R,6S,3'R)-5,6-Dihydro-β,β-carotene-3,5,6,3'-tetrol*, **5**): Red crystals. M.p. 145–147°. UV/VIS (benzene): 487, 457, 434. No furanoid reaction. CD (EPA, r.t.): 203 (+ 0.30), 205 (+ 0.84), 213 (0.00), 241 (+ 0.58), 276 (−0.92); CD (EPA, −180°): 202 (+ 0.46), 205 (+ 0.85), 217 (−0.46), 242 (+ 1.53), 280 (−3.15), 459 (+ 0.35). ^{1}H-NMR ($CDCl_3$): 0.82 (*s*, Me(16)); 1.07 (*s*, Me(16')); 1.07 (*s*, Me(17')); 1.10 (*s*, Me(18)); 1.26 (*s*, Me(17)); 1.48 (*Ψt*, $J_{gem} \approx J(2'ax,3') = 12.1$, H_{ax}–C(2')); 1.53 (*m*, H_{ax}–C(2)); 1.63 (*m*, $J_{gem} \approx 14$, H_{ax}–C(4)); 1.74 (*s*, Me(18')); 1.77 (*ddd*, $J_{gem} = 12.1$, $J(2'eq,3') = 3.4$, $J(2'eq,4'eq) \approx 2$, H_{eq}–C(2')); 1.78 (*m*, H_{eq}–C(2)); 1.92 (*s*, Me(19)); 1.96 (*s*, Me(19')); 1.96 (*s*, Me(20')); 1.97 (*s*, Me(20)); 2.04 (*dd*, $J_{gem} = 16.6$, H_{ax}–C(4')); 2.11 (*m*, $J_{gem} \approx 14$, H_{eq}–C(4)); 2.39 (*ddd*, $J_{gem} = 16.6$, $J(4'eq,3') \approx 6$, H_{eq}–C(4')); 4.00 (*m*, H–C(3')); 4.27 (*m*, H–C(3)); 5.87 (*d*, $J(7,8) = 15.8$, H–C(7)); *ca.* 6.10 (*m*, $J(7',8') = 16.7$, H–C(7')); *ca.* 6.10 (*m*, $J(8',7') = 16.7$, H–C(8')); 6.16 (*d*, $J(10',11') = 12.2$, H–C(10')); 6.22 (*d*, $J(10,11) = 10.7$, H–C(10)); 6.25 (*m*, H–C(14)); 6.26 (*m*, H–C(14')); 6.36 (*d*, $J(12',11') = 14.9$, H–C(12')); 6.37 (*d*, $J(12,11) = 14.9$, H–C(12)); 6.42 (*d*, $J(8,7) = 15.8$, H–C(8)); 6.60 (*dd*, $J(11,10) = 10.7$, $J(11,12) = 14.9$, H–C(11)); 6.63 (*m*, H–C(15)); 6.63 (*m*, H–C(15')); 6.64 (*dd*, $J(11',10') = 12.2$, $J(11',12') = 14.9$, H–C(11')). EI-MS: 602 (77, M^+), 584 (57, $[M - H_2O]^+$), 566 (10, $[M - 2\,H_2O]^+$), 504 (32, $[M$ − toluene$]^+$), 221 (65), 181 (50), 145 (78), 119 (91), 105 (65), 55 (44), 45 (100).

4. *5,6-Diepikarpoxanthin* (*(3S,5S,6S,3'R)-5,6-Dihydro-β,β-carotene-3,5,6,3'-tetrol*, **13**): Red crystals. M.p. 150°. UV/VIS (benzene): 487, 458, 434. No furanoid reaction. CD (EPA, r.t.): 208 (−1.95), 248 (+ 1.13), 283 (−1.75). CD (EPA, −180°): 212 (−0.23), 220 (−1.32), 247 (+ 3.06), 281 (−6.89), 311 (−0.73), 332 (−1.19), 466 (−0.31), 491 (+ 0.47), 498 (−0.92). ^{1}H-NMR ($CDCl_3$): 0.89 (*s*, Me(16)); 1.07 (*s*, Me(16')); 1.07 (*s*, Me(17')); 1.12 (*s*, Me(18)); 1.31 (*s*, Me(17)); 1.47 (*Ψt*, $J_{gem} \approx J(2'ax,3') = 11.9$, H_{ax}–C(2')); 1.65 (*ddd*, $J_{gem} = 14.7$, $J(2ax,3) = 3.3$, $J(2ax,4ax) = 2.1$, H_{ax}–C(2)); 1.73 (*s*, Me(18')); 1.77 (*ddd*, $J_{gem} = 11.9$, $J(2'eq,3') = 3.6$, $J(2'eq,4'eq) = 2.1$, H_{eq}–C(2')); 1.84 (*ddd*, $J_{gem} = 14.7$, $J(4ax,3) = 2.8$, $J(4ax,2ax) = 2.1$, H_{ax}–C(4)); 1.89 (*dd*, $J_{gem} = 14.7$, $J(2eq,3) = 3.3$, H_{eq}–C(2)); 1.97 (*s*, Me(19')); 1.97 (*s*, Me(20')); 1.97 (*s*, Me(20)); 1.98 (*s*, Me(19)); 2.04

(*dd*, J_{gem} = 16.9, *J*(4'ax,3') = 9.9, H_{ax}–C(4')); 2.10 (*dd*, J_{gem} = 14.7, *J*(4eq,3) = 3.4, H_{eq}–C(4)); 2.38 (*ddd*, J_{gem} = 16.9, *J*(4'eq,3') = 5.8, *J*(4'eq,2'eq) = 2.1, H_{eq}–C(4')); 4.00 (*m*, H–C(3')); 4.27 (*m*, H–C(3)); *ca.* 6.11 (*m*, *J*(7',8') = 17.0, H–C(7')); *ca.* 6.11 (*m*, *J*(8',7') = 17.0, H–C(8')); 6.15 (*d*, *J*(10',11') = 11.3, H–C(10')); 6.22 (*d*, *J*(10,11) = 11.7, H–C(10)); 6.26 (*m*, H–C(14)); 6.26 (*m*, H–C(14')); 6.35 (*m*, H–C(7)); 6.35 (*m*, H–C(8)); 6.36 (*d*, *J*(12',11') = 14.7, H–C(12')); 6.37 (*d*, *J*(12,11) = 15.1, H–C(12)); 6.63 (*dd*, *J*(11,10) = 11.7, *J*(11,12) = 15.1, H–C(11)); 6.63 (*m*, H–C(15)); 6.63 (*m*, H–C(15')); 6.64 (*dd*, *J*(11,10') = 11.3, *J*(11',12') = 14.7, H–C(11')). ^{13}C-NMR ($CDCl_3$)[1]: 12.74 (C(19')); 12.80 (C(20')); 12.80 (C(20)); 13.33 (C(19)); 21.61 (C(18')); 26.02 (C(18)); 27.53 (C(16)); 28.29 (C(17)); 28.71 (C(16')); 30.25 (C(17')); 37.11 (C(1')); 37.97 (C(1)); 39.58 (C(4)); 42.53 (C(4')); 43.06 (C(2)); 48.40 (C(2')); 65.08 (C(3')); 68.96 (C(3)); 76.23 (C(5)); 79.73 (C(6)); 124.81 (C(11)); 124.97 (C(11')); 125.58 (C(7')); 126.16 (C(5')); 130.02* (C(15')); 130.15* (C(15)); 130.19 (C(7)); 131.29 (C(10')); 131.64 (C(10)); 132.56 (C(14')); 132.71 (C(14)); 134.20 (C(8)); 134.83 (C(9)); 135.70 (C(9')); 136.39 (C(13)); 136.53 (C(13')); 137.54 (C(12')); 137.74 (C(6')); 137.83 (C(12)); 138.48 (C(8')). EI-MS: 602 (37, M^+), 584 (96, $[M - H_2O]^+$), 566 (10, $[M - H_2O]^+$), 504 (49, $[M$ – toluene$]^+$), 221 (100), 181 (65), 145 (21), 119 (27), 91 (35).

5. *5,6-Diepilutoxanthin* (*3S,5S,6S,3'S,5'R,6'S)-5',6'-Epoxy-5,6,5',6'-tetrahydro-β,β-carotene-3,5,6,3'-tetrol*, **14**): Orange crystals. M.p. 155–157°. TLC: R_f 0.37. UV/VIS (benzene): 483, 453, 427. After acid treatment: 460, 431, 407. CD (EPA, r.t.): 234 (–0.13), 267 (–3.65), 328 (+0.49). CD (EPA, –180°): 209 (+1.47), 227 (–0.43), 245 (+0.78), 268 (–2.85), 283 (–0.79), 305 (+0.16). ^{1}H-NMR ($CDCl_3$)[2]): 0.89 (*s*, Me(16)); 0.98 (*s*, Me(16')); 1.12 (*s*, Me(18)); 1.15 (*s*, Me(17')); 1.19 (*s*, Me(18')); 1.24 (*m*, H_{ax}–C(2')); 1.31 (*s*, Me(17)); 1.61 (*m*, H_{eq}–C(2')); 1.63 (*m*, $J_{gem} \approx 13.2$, H_{ax}–C(4')); 1.65 (*ddd*, $J_{gem} \approx 15$, H_{ax}–C(2)); 1.85 (*ddd*, J_{gem} = 14.5, *J*(4ax,3) ≈ 3, H_{ax}–C(4)); 1.90 (*dd*, $J_{gem} \approx 15$, *J*(2eq,3) = 3.4, H_{eq}–C(2)); 1.93 (*s*, Me(19')); 1.96* (*s*, Me(20)); 1.97* (*s*, Me(20')); 1.98 (*s*, Me(19)); 2.10 (*dd*, J_{gem} = 14.5, *J*(4eq,3) = 3.5, H_{eq}–C(4)); 2.38 (*ddd*, J_{gem} = 13.2, *J*(4'eq,3') = 3.1, *J*(4'eq,2'eq) ≈ 2, H_{eq}–C(4')); 3.91 (*m*, H–C(3')); 4.27 (*m*, H–C(3)); 5.88 (*d*, *J*(7',8') = 15.6, H–C(7')); 6.18 (*d*, *J*(10',11') = 11.1, H–C(10')); 6.21 (*d*, *J*(10,11) = 10.9, H–C(10)); *ca.* 6.27 (*m*, H–C(14')); *ca.* 6.27 (*m*, H–C(14)); 6.29 (*d*, *J*(8',7') = 15.6, H–C(8')); *ca.* 6.35 (*m*, H–C(7)); *ca.* 6.35 (*m*, H–C(8)); 6.37 (*d*, *J*(12,11) = 14.9, H–C(12)); 6.37 (*d*, *J*(12',11') = 14.9, H–C(12')); 6.60 (*dd*, *J*(11',10') = 11.1, *J*(11',12') = 14.9, H–C(11')); *ca.* 6.63 (*m*, H–C(15)); *ca.* 6.63 (*m*, H–C(15')); 6.64 (*dd*, *J*(11,10) = 10.9, *J*(11,12) = 14.9, H–C(11)). EI-MS: 618 (32, M^+), 600 (15, $[M - H_2O]^+$), 526 (9, $[M$ – toluene$]^+$), 287 (28), 221 (71), 181 (35), 145 (40), 119 (35), 91 (100).

6. *5,6-Diepicapsokarpoxanthin* (*3S,5S,6S,3'S,5'R)-5,6-Dihydro-3,5,6,3'-tetrahydroxy-β,κ-caroten-6'-one*; **15**): Red crystals. M.p.: 208–210°. TLC: R_f 0.48. UV/VIS (benzene): 505 (sh), 480. CD (EPA, r.t.): 202 (–20.77), 211 (–6.71), 240 (–22.78), 282 (+5.36). CD (EPA, –180°): 204 (–50.28), 208 (+3.52), 240 (–26.70), 286 (+11.50). ^{1}H-NMR ($CDCl_3$): 0.84 (*s*, Me(16')); 0.89 (*s*, Me(16)); 1.12 (*s*, Me(18)); 1.20 (*s*, Me(17')); 1.31 (*s*, Me(17)); 1.37 (*s*, Me(18')); 1.48 (*dd*, J_{gem} = 14.5, *J*(4'ax,3') = 3.2, H_{ax}–C(4')); 1.65 (*ddd*, J_{gem} = 14.7, *J*(2ax,3) = 3.6, *J*(2ax,4ax) = 2.2, H_{ax}–C(2)); 1.71 (*dd*, J_{gem} = 13.6, *J*(2'ax,3') = 4.5, H_{ax}–C(2')); 1.84 (*ddd*, J_{gem} = 14.5, *J*(4ax,3) = 2.5, *J*(4ax,2ax) = 2.2, H_{ax}–C(4)); 1.90 (*dd*, J_{gem} = 14.7, *J*(2eq,3) = 3.7, H_{eq}–C(2)); 1.956 (*s*, Me(19')); 1.975 (*s*, Me(20')); 1.987 (*s*, Me(20)); 1.989 (*s*, Me(19)); 1.99 (*dd*, J_{gem} = 13.6, *J*(2'eq,3') = 8.5, H_{eq}–C(2')); 2.10 (*dd*, J_{gem} = 14.5, *J*(4eq,3) = 3.2, H_{eq}–C(4)); 2.96 (*dd*, J_{gem} = 14.5, *J*(4'eq,3') = 8.5, H_{eq}–C(4')); 4.28 (*m*, H–C(3)); 4.51 (*m*, H–C(3')); 6.22 (*d*, *J*(10,11) = 11.6, H–C(10)); 6.27 (*d*, *J*(14,15) = 11.2, H–C(14)); 6.36 (*d*, *J*(14',15') not determined due to strong signal overlap, H–C(14')); 6.36 (*m*, H–C(7)); 6.36 (*m*, H–C(8)); 6.38 (*d*, *J*(12,11) = 14.9, H–C(12)); 6.44 (*d*, *J*(7',8') = 15.1, H–C(7')); 6.52 (*d*, *J*(12',11') = 14.6, H–C(12')); 6.55 (*d*, *J*(10',11') = 10.7, H–C(10')); 6.62 (*dd*, *J*(11',10') = 10.7, *J*(11',12') = 14.6, H–C(11')); 6.64 (*m*, H–C(15')); 6.67 (*dd*, *J*(11,10) = 11.6, *J*(11,12) = 14.9, H–C(11)); 6.70 (*m*, H–C(15)); 7.33 (*d*, *J*(8',7') = 15.1, H–C(8')). ^{13}C-NMR ($CDCl_3$): 12.73 (C(19')); 12.85 (C(20')); 12.85 (C(20)); 13.37 (C(19)); 21.27 (C(18')); 25.08 (C(17')); 25.85 (C(16')); 26.05 (C(18)); 27.53 (C(16)); 28.30 (C(17)); 37.97 (C(1)); 39.85 (C(4)); 43.08 (C(2)); 43.96 (C(1')); 45.30 (C(4')); 50.85 (C(2')); 58.93 (C(5')); 68.99 (C(3)); 70.38 (C(3')); 76.18 (C(5)); 79.74 (C(6)); 120.88 (C(7')); 124.11 (C(11')); 125.39 (C(11)); 129.75 (C(15')); 130.50 (C(7)); 131.56 (C(10)); 131.56 (C(15)); 132.48 (C(14)); 133.66 (C(9')); 134.16 (C(8)); 135.08 (C(9)); 135.27 (C(14')); 135.95 (C(13)); 137.50 (C(12)); 137.66 (C(13')); 140.67 (C(10')); 141.94 (C(12')); 146.85 (C(8')); 202.90 (C(6')). EI-MS: 618 (7, M^+), 600 (19, $[M - H_2O]^+$), 582 (5, $[M - 2\,H_2O]^+$), 494 (32), 221 (63), 181 (36), 145 (47), 119 (28), 109 (100), 91 (62), 83 (51), 43 (29).

Derivatization. Reduction of **15** with $NaBH_4$ yielded a mixture of epimers of 5,6-dihydro-β,κ-carotene-3,5,6,3',6'-pentols. UV/VIS (benzene): 481, 451, 426. TLC: R_f 0.28. EI-MS: 620 (9, M^+), 602 (2, $[M - H_2O]^+$), 584 (5, $[M - 2\,H_2O]^+$), 221 (33), 181 (43), 145 (59), 109 (70), 109 (69), 91 (100), 83 (66).

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[1]) *: Assignments may be interchanged.

Acetylation of **15** yielded 3,3′-diacetyl-5,6-dihydro-5,6-dihydroxy-β,κ-caroten-6′-one: UV/VIS (benzene): 505 (sh), 480. TLC: R_f 0.78. EI-MS: 702 (10, M^+), 642 (18), 263 (23), 221 (26), 181 (25), 145 (37), 109 (100), 91 (52), 83 (38), 43 (50).

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