120. Reisolation of Carotenoid 3,6-Epoxides from Red Paprika (*Capsicum annuum*)

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Cucurbitaxanthin A (= (3*S*,5*R*,6*R*,3′*R*)-3,6-epoxy-5,6-dihydro-β,β-carotene-5,3′-diol; **5**), cucurbitaxanthin B (= (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-3,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol; **6**), the epimeric cucurbitachromes 1 and 2 (= (3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*S*)- and (3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*R*)-3,6,5′,8′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol, resp.; **9**/**10**), cycloviolaxanthin (= (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*R*)-3,6,3′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,5′-diol; **8**), and capsanthin 3,6-epoxide (= (3*S*,5*R*,6*R*,3′*S*,5′*R*)-3,6-epoxy-5,6-dihydro-5,3′-dihydroxy-β,κ-caroten-6′-one; **7**) were isolated from red spice paprika (*Capsicum annuum* var. *longum*) and characterized by their ^{1}H- and ^{13}C-NMR, mass, and CD spectra.

1. Introduction. – The different varieties of paprika (*Capsicum annuum*) have been investigated for a long time. It has been established that capsanthin (**1**) and capsorubin, 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 3,5,6-trihydroxy-β-end group (karpoxanthin (**2**)), 3,4-didehydro-6-hydroxy-γ-end group (nigroxanthin (**3**)), and oxabicyclo-β-end group have been isolated [1] [2].

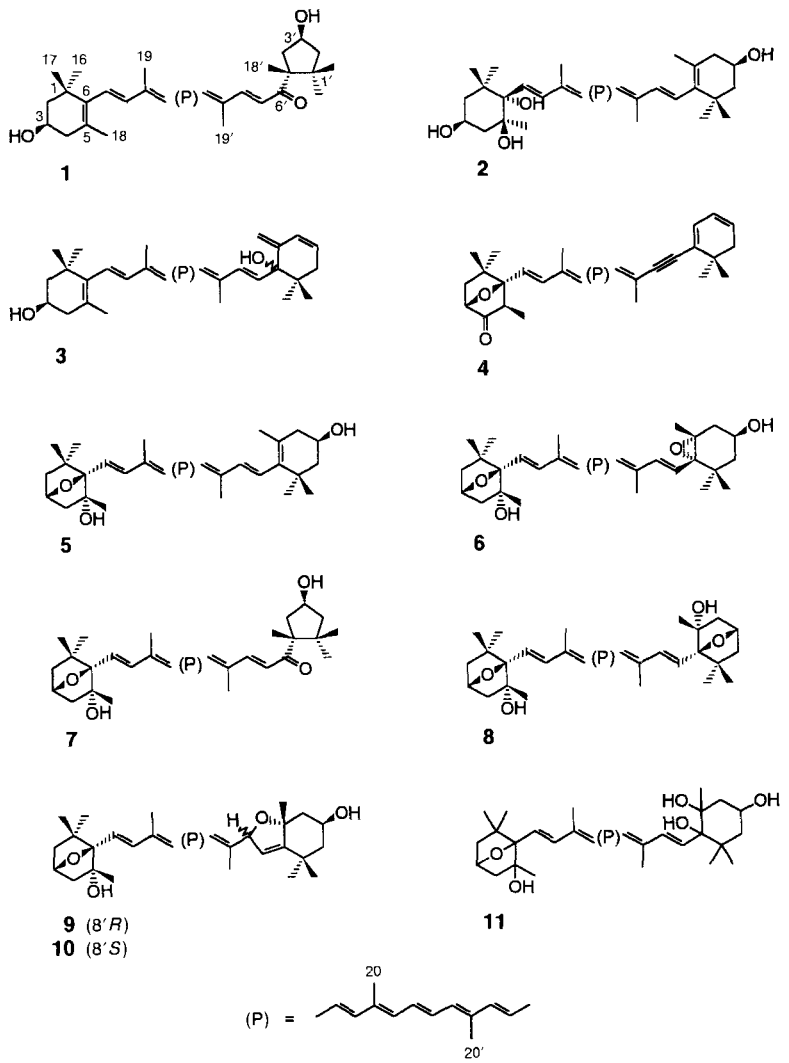
The naturally occurring carotenoids with a 7-oxabicyclo[2.2.1]heptane end group (= 3,6-epoxy-5,6-dihydro-β-end group), eutreptiellanone (**4**), α-cryptoeutreptiellanone, and β-cryptoeutreptiellanone, were first isolated from the marine alga *Eutreptiella gymnastica* [3–5]. The separation of cucurbitaxanthin A (**5**) and B (**6**) out of pumpkin [6], and of cucurbitaxanthin A (**5**) and capsanthin 3,6-epoxide (**7**) out of red paprika succeeded at the same time [1]. A few years later, cycloviolaxanthin (**8**) and cucurbitaxanthin B (**6**) could be isolated from black paprika [7–9]. An attempt to synthesize cycloviolaxanthin (**8**) was performed, too [10], and the semisynthetic cucurbitaxanthin B (**6**) was obtained from cucurbitaxanthin A (**5**) by epoxidation [9].

The compounds containg the 3,5,6-trihydroxy-β-end group, the 3,4-didehydro-6-hydroxy-κ-end group, and the 3,6-epoxy-5,6-dihydro-β-end group may be formed from antheraxanthin and violaxanthin, and their occurrence is interrelated with the biosynthesis of the κ-end group, which has not yet been clarified in every detail. The probable biosynthetic route of the formation of the 3,6-epoxy-end group is as follows: the hydrolytic opening of the 5,6-epoxy ring results in 3,5,6-trihydroxy compound, which yields the 3,6-epoxy-end group in compliance with Scheme 2 in [10]. Therefore, cucurbitaxanthin A (**5**) may be formed from antheraxanthin, cucurbitaxanthin B (**6**) and cycloviolaxanthin (**8**) from violaxanthin, and capsanthin 3,6-epoxide (**7**) from capsanthin

5,6-epoxide. However, an alternative biosynthetic pathway is conceivable: the conversion of cucurbitaxanthin A (**5**) to cucurbitaxanthin B (**6**) by epoxidation and the following pinacol rearrangement to capsanthin 3,6-epoxide (**7**). Considering the furanoid-oxide rearrangement and the hydrolysis, the epimeric cucurbitachromes 1 and 2 (**9**/**10**) and 3,6-epoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′,5′,6′-tetrol (**11**) may be formed from cucurbitaxanthin B, too.

Hitherto, four carotenoids containing the 3,6-epoxy-β-end group, *i.e.*, **5**–**8**, have been isolated from paprika [1] [7] [9]; however, as some of the spectroscopic data are missing, they are included in these current investigations.

As a continuation of our work on paprika carotenoids, we aimed at the isolation of all existing carotenoids containing the 3,6-epoxy-end group. In this paper, we describe a



new isolation method for 3,6-epoxides from paprika, namely, for cucurbitaxanthin A (**5**) and B (**6**), capsanthin 3,6-epoxide (**7**), cycloviolaxanthin (**8**), and the epimeric cucurbitachromes 1 and 2 (**9**/**10**), as well as their spectroscopic analysis.

2. Results. – *Isolation.* The new isolation method for 3,6-epoxides from red paprika (*Capsicum annuum* var. *longum*) is, in essence, based on the distribution and fractionated precipitation. The fresh ripe paprika pods were first extracted with MeOH, then with Et_2O. After the saponification, the MeOH fraction contained the target carotenoids, besides a number of different secondary compounds, which could be separated mostly by distribution and sequential precipitation steps. A detailed description of the procedure is given in the *Exper. Part.* The addition of benzene/hexane to the pretreated MeOH mother liquor caused a precipitation of a crystalline carotenoid mixture (150 mg) which could be separated on a $CaCO_3$ column (benzene/hexane 2:3) into the following fractions: *cis*-capsanthins (*1*), mixture of karpoxanthin and luteoxanthin (*2*), capsanthin 3,6-epoxide (*3*), mixture of cucurbitaxanthin B, cucurbitachromes, capsanthin, and mutatoxanthin (*4*), mixture of nigroxanthin and capsanthone (*5*), cucurbitaxanthin A (*6*), and cycloviolaxanthin (*7*).

Rechromatography of *Fractions 3*, *4*, *6*, and *7* yielded 20 mg of capsanthin 3,6-epoxide (**7**), 2 mg of cucurbitaxanthin B (**6**), 1 mg of cucurbitachrome 2 (**10**), 8 mg of cucurbitaxanthin A (**5**), and 3 mg of cycloviolaxanthin (**8**), respectively. In addition, the epimeric cucurbitachrome 1 (**9**) and the 3,6-epoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′,5′,6′-tetrol (**11**) could be isolated in very small amounts. The structural analysis of the compounds **5**–**11** was performed by mass, NMR, UV/VIS, and CD spectroscopy.

Spectroscopical Characterization. In every case, the MS showed the corresponding molecular-ion peaks. In addition to the signals typical for carotenoids ($[M - H_2O]^+$, $[M$ – methylcyclopentadiene$]^+$, $[M$ – toluene$]^+$), strong peaks at m/z 286, 221, 160, 155, and 43 characterizing the 3,6-epoxy-end group were observed.

At present, no NMR data are available for compound **11**; the results of UV/VIS, and MS measurements, however, confirmed its constitution. The NMR data of the 3,6-epoxy carotenoids **5**–**10** were completely in accordance with their presented constitutions and configurations. ^{1}H-NMR and ^{1}H,^{1}H-COSY experiments allowed complete ^{1}H-signal assignments, and the δ(H) and J(H,H) values of the end groups were identical with the corresponding data from the literature [9] [11–13]. With the exception of (8′*R*)-cucurbitachrome 1 (**9**), where the sample amount was not sufficient for any ^{13}C-NMR

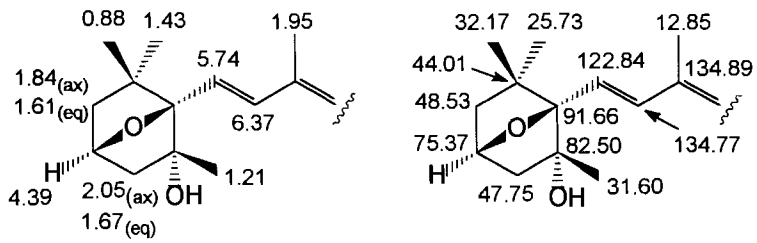


Fig. 1. *^{1}H- and ^{13}C-NMR chemical shifts* [ppm] *for the 3,6-epoxy-end group in cycloviolaxanthin* (**8**)

analysis, and cucurbitaxanthin B (**6**), where ^{13}C-NMR data could be extracted only from traces of an inverse HMQC experiment and, as a consequence, no δ(C) information of quaternary C-atoms were available, ^{13}C-line assignments were successfully achieved by means of ^{13}C, DEPT-135, and inverse HMQC experiments. The ^{13}C-NMR data of the 3,6-epoxy-end group were identical with the data found for cucurbitaxanthin A (**5**) originating from pumpkin [6] and for 3,6-epoxy-7-megastigmene-5,9-diol originating from Greek tobacco [14], except the signals of C(2) and C(4) [6]. However, ^{13}C,^{1}H-shift-correlation experiments with cycloviolaxanthin (**8**) allowed the unambiguous assignment of C(2) to the ^{13}C-signal at 48.53 ppm and of C(4) to that at 47.75 ppm (see *Fig. 1*). The ^{13}C-NMR data of the 3-hydroxy-β-, the 5,6-epoxy-3-hydroxy-β-, the 5,8-epoxy-3-hydroxy-β-, and the κ-end groups in **5**–**7** and **10** were in agreement with published results [11–13].

The quantitative CD spectrum (*Fig. 2*) of cycloviolaxanthin (**8**) confirmed the (3*S*,5*R*,6*R*)-configuration of the 3,6-epoxy-end group reported in the previous paper [7]. Contrasting the positive curve in the CD spectrum of **8** at 270 nm to the negative curve in the CD spectra of violaxanthin [14] [15] or of (5*R*,6*S*,5′*R*,6′*S*)-5,6,5′,6′-diepoxy-β,β-carotene [16] [17] demonstrates that the configuration at C(6) of **8** is changed. Since the CD spectrum of cycloviolaxanthin has a weak *Cotton* effect, the CD spectra of the other 3,6-epoxy compounds, *i.e.*, **5**–**7** and **10**, show a strong influence of the zeaxanthin-, violaxanthin-, κ-, and 5,8-epoxy-end groups.

4. Discussion. – The isolation of the 3,6-epoxides from red paprika helps to clarify the biosynthesis of paprika carotenoids. On the one hand, the cycloviolaxanthin (**8**), the capsanthin 3,6-epoxide (**7**), and the epimeric cucurbitachromes 1 and 2 (**9**/**10**) represent end products, while the cucurbitaxanthin A (**5**) is likely to be the precursor of cucurbitaxanthin B (**6**); and, on the other hand, the cucurbitaxanthin B (**6**) is likely to be the precursor of both cycloviolaxanthin (**8**) and capsanthin 3,6-epoxide (**7**). However, it has not been clarified yet if there is an interrelation between the formation of the κ-end group from 5,6-epoxides and the formation of 3,6-epoxides from 5,6-epoxides. In a previous paper [18], we reported that carotenoids with κ-, 3,5,6-trihydroxy-, and 3,6-epoxy-end groups could not be detected in the yellow paprika (*C. annuum lycopersiciforme flavum*, cv. SZENTESI sárga paradicsom paprika). In this kind of paprika, the main carotenoids are carotenoid 5,6-epoxides, (violaxanthin and antheraxanthin), and the colour of this variety never turns red. In contrast with this result, in all other varieties, whose colour turns red during ripening, carotenoids with κ-end group are formed, and we always could detect the 3,6-epoxy-carotenoids as minor components [8] [19]. Therefore, we assume that the occurrence of the 3,6-epoxycarotenoids in red paprika is related to the formation of the κ-end group; these 3,6-epoxycarotenoids may be the by-product of the formation of capsanthin and capsorubin.

The mechanism of the formation of the 3,6-epoxy-end group from the 5,6-epoxy-end group is questionable, too. A lot of flowers containing 5,6-epoxy- and 5,8-epoxy-carotenoids have been investigated, and through some 3,5,6-trihydroxycarotenoids could be isolated [20], the detection of 3,6-epoxycarotenoids have failed. Although the reaction of a 5,6-epoxy-3-hydroxy-end group with dilute acid may produce the 3,5,6-trihydroxy [20], and 3,6-epoxy compounds [14] [21] in addition to the furanoid 5,8-epoxides, we assume that the formation of 3,6-epoxycarotenoids in plants is an enzymatic effect. The confirmation of this assumption demands further biochemical investigations.

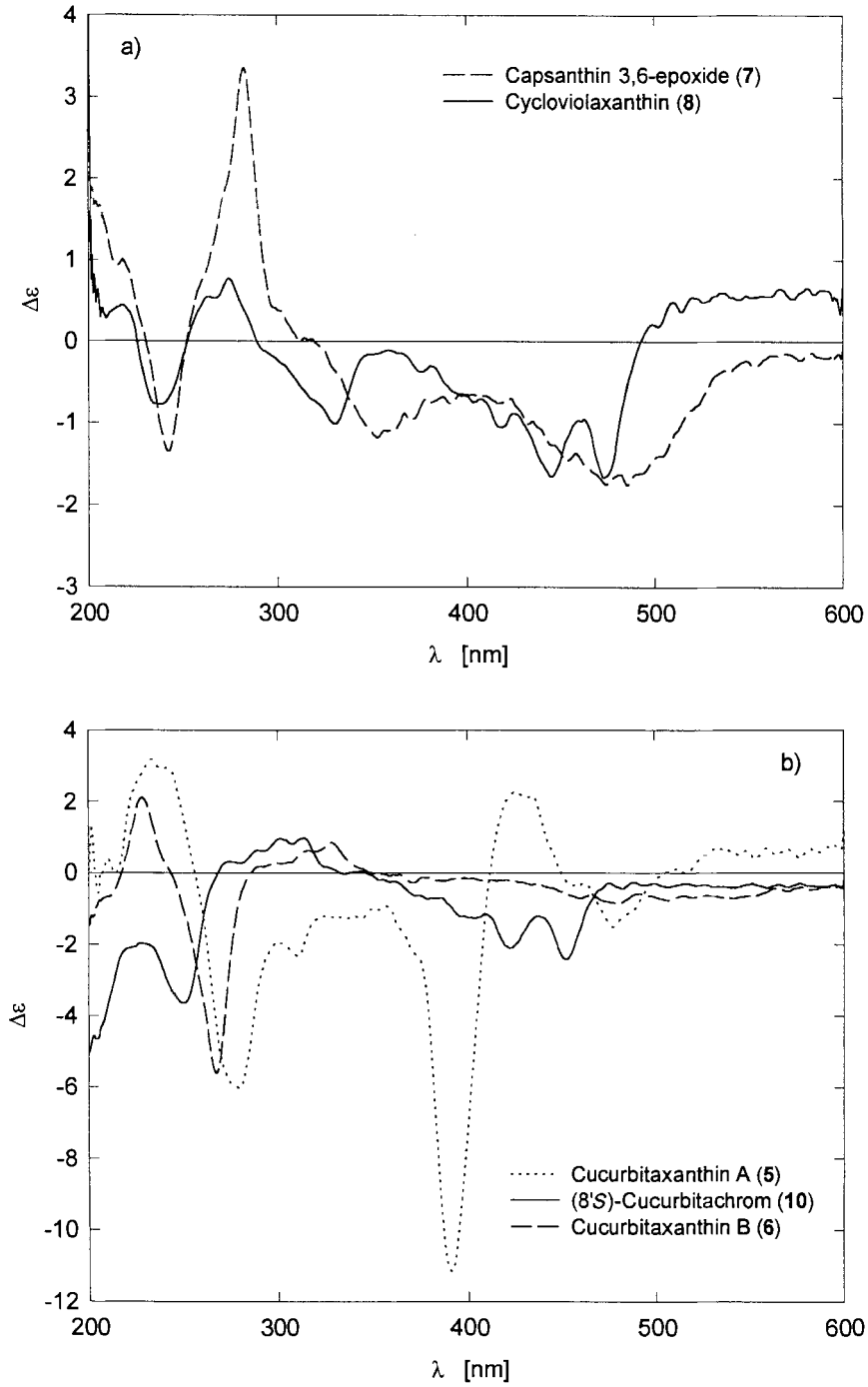


Fig. 2. *CD Spectra of carotenoid 3,6-epoxides in EPA* (Et_2O/isopentane/EtOH 5:5:2) *at room temperature*

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Experimental Part

1. *General.* HPLC: *Gynkotek* pump model *300 B* with *Gynkotek* gradient former; *Waters-991* detector, photo diode array; column: 250 × 4.6 mm i.d., *Chromsil* C_{18} (6 μm), end-capped; gradient elution with *A* (12% H_2O/MeOH), *B* (MeOH), and *C* (acetone/MeOH 1:1), *i.e.* 0–2 min 100% *A*, 2–10 min→*A*/*B* 4:1, 10–18 min→*A*/*B* 1:1, 18–25 min→100% *B*, 25–27 min 100% *B*, 27–34 min→100% *C*, 34–41 min 100% *C* (linear steps). UV/VIS: *Beckman DU-65*; in nm. CD: *Jobin-Yvon Dichrograph-6*; EPA = Et_2O/isopentane/EtOH 5:5:2; $\lambda(\Delta\varepsilon)$ in nm. NMR: *Bruker AM 400* and *Bruker DRX 400* (^1H: 400.14 MHz; ^{13}C: 100.61 MHz); in $CDCl_3$ at 20°; chemical shifts δ in ppm (rel. to the solvent signal), coupling constants *J* in Hz. MS: *Varian MA-CH 7A*; *m*/*z* (rel. intensity in %).

2. *Isolation of 3,6-Epoxides.* Ripe red-paprika pods (*Capsicum annuum* var. *longum*), freed from seeds and stem (2.3 kg of fresh weight), were used for extraction. The material was blended with MeOH and *ca.* 1% $CaCO_3$ soln. The blend was allowed to stand in MeOH for dehydration. After 20 h, the mixture was filtered and the filter cake extracted repeated twice with MeOH (*a*) and finally with Et_2O (*b*).

The three MeOH extracts (*a*) were combined, diluted with Et_2O, washed free from MeOH with H_2O, dried (Na_2SO_4), concentrated under vacuum to about half-volume, and saponified with 30% KOH/MeOH at r.t. for 18 h. After saponification, the Et_2O soln. was washed free from alkali and evaporated and the residue distributed between hexane and MeOH/H_2O 9:1. The epiphasic pigments were precipitated from benzene/MeOH (92 mg). The hypophasic pigments were precipitated from benzene/hexane (450 mg). The mother liquor was precipitated again from benzene with hexane (150 mg). This crystalline pigments were separated by column chromatography.

The Et_2O extract (*b*) was washed free from MeOH, concentrated under vacuum to about half-volume, and saponified with 30% KOH/MeOH at r.t. for 18 h. After saponification, the Et_2O soln. was washed free from alkali, and evaporated and the residue dissolved in benzene. The hypophasic pigments were precipitated with hexane (1.90 g). The mother liquor was evaporated and the residue dissolved in benzene, and the epiphasic pigments were precipitated with MeOH (200 mg). The mother liquor was distributed between hexane and MeOH/H_2O 9:1. The epiphasic pigments were dissolved in benzene and precipitated with MeOH (50 mg). The hypophasic pigments were dissolved in benzene and precipitated with hexane (81 mg).

3. *Separation of the 3,6-Epoxides.* The separation of the 150 mg of crystalline mixture was achieved by column chromatography on $CaCO_3$ (*Biogal*) with hexane/benzene 3:2. The following fractions were obtained: *Fr. 1*: unidentified and *cis*-capsanthins; *Fr. 2*: mixture of karpoxanthin (UV/VIS (benzene): 487, 457, 434) and luteoxanthin (UV/VIS (benzene): 460, 432, 408; UV (after acid treatment): 436, 410, 388); *Fr. 3*: capsanthin 3,6-epoxide (**7**); *Fr. 4*: cucurbitaxanthin **B** (**6**), cucurbitachromes, capsanthin, and mutatoxanthin; *Fr. 5*: mixture of nigroxanthin (UV/VIS (benzene): 487, 457, 434; no furanoid reaction), capsanthone (UV/VIS (benzene): 493), and *cis*-cucurbitaxanthin A (UV/VIS (benzene): 485, 454); *Fr. 6*: cucurbitaxanthin A (**5**); *Fr. 7*: cycloviolaxanthin (**8**).

Fr. 3, *6*, and *7* were rechromatographed ($CaCO_3$) and crystallized from benzene/hexane: 20 mg of **7**, 8 mg of **5**, and 3 mg of **8**, resp.

Rechromatography ($CaCO_3$, benzene/hexane 3:2) of *Fr. 4* resulted in the following fractions: *Fr. 4.1*: unidentified; *Fr. 4.2*: cucurbitachromes **9**/**10**; *Fr. 4.3*: capsanthin (UV/VIS (benzene): 505, 485); *Fr. 4.4*: mutatoxanthin (UV/VIS (benzene): 465, 438); *Fr. 4.5*: cucurbitaxanthin **B** (**6**). *Fr. 4.5* was rechromatographed ($CaCO_3$) and crystallized from benzene/hexane: 2 mg of **6**.

Rechromatography ($CaCO_3$, benzene/hexane 3:2) of *Fr. 4.2* resulted in the following fractions: *Fr. 4.2.1*: cucurbitachrome 1 (**9**; UV/VIS (benzene): 460, 432, 408; no furanoid reaction); *Fr. 4.2.2*: unidentified; *Fr. 4.2.3*: cucurbitachrome 2 (**10**) and unidentified; *Fr. 4.2.4*: unidentified. *Fr. 4.2.1* was crystallized from benzene/hexane: *ca.* 0.1 mg of **9**. Rechromatography ($CaCO_3$, acetone/hexane 3:97) of *Fr. 4.2.3* resulted in the following fractions: *Fr. 4.2.3.1*: unidentified (UV/VIS (benzene): 483, 453, 427; no furanoid reaction); *Fr. 4.2.3.2*: cucurbitachrome 2 (**10**). *Fr. 4.2.3.2* was crystallized from benzene/hexane: *ca.* 1 mg of **10**. *Fr. 4.2.3.1* was crystallized from benzene/hexane: *ca.* 0.05 mg of 3,6-epoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′,5′,6′-tetrol (**11**).

4. *Cucurbitaxanthin A* (= *(3S,5R,6R,3′R)-3,6-Epoxy-5,6-dihydro-β,β-carotene-5,3′-diol*; **5**): Orange crystals. M.p. 160–162°. UV/VIS (benzene): 487, 457, 434. CD (EPA, r.t.): 230 (+3.08), 278 (−6.15), 390 (−11.32), 426

(+2.38), 476 (−1.51). CD (EPA, −180°): 214 (−9.61), 236.5 (+9.26), 279 (−21.49), 390.5 (−8.55), 437 (+2.19), 495 (−2.14). ^{1}H-NMR ($CDCl_3$): 0.88 (*s*, Me(17)); 1.07 (*s*, Me(16′), Me(17′)); 1.21 (*s*, Me(18)); 1.43 (*s*, Me(16)); 1.48 (‘*t*’, $J_{gem} \approx J(2'ax,3') \approx 12.0$, H_{ax}–C(2′)); 1.61 (*d*, $J_{gem} = 11.5$, H_{eq}–C(2)); 1.67 (*d*, $J_{gem} = 12.1$, H_{eq}–C(4)); 1.73 (*s*, Me(18′)); 1.77 (*ddd*, $J_{gem} = 12.0$, $J(2'eq,3') = 3.6$, $J(2'eq,4'eq) = 2.1$, H_{eq}–C(2′)); 1.84 (*ddd*, $J_{gem} = 11.5$, $J(2ax,3) = 6.0$, $J(2ax,4ax) = 2.2$, H_{ax}–C(2)); 1.95 (*s*, Me(19)); 1.96 (*s*, Me(20), Me(19′), Me(20′)); 2.04 (*dd*, $J_{gem} = 16.1$, $J(4'ax,3') = 9.8$, H_{ax}–C(4′)); 2.06 (*ddd*, $J_{gem} = 12.1$, $J(4ax,3) = 6.0$, $J(4ax,2ax) = 2.2$, H_{ax}–C(4)); 4.00 (*m*, H–C(3′)); 4.39 (‘*t*’, $J(3,2ax) \approx J(3,4ax) = 6.0$, H–C(3)); 5.74 (*d*, $J(7,8) = 16.0$, H–C(7)); 6.09 (*AB*, $J(7',8') = 16.8$, H–C(7′)); 6.14 (*AB*, $J(8',7') = 16.8$, H–C(8′)); 6.15 (*d*, $J(10',11') = 11.3$, H–C(10′)); 6.20 (*d*, $J(10,11) = 11.4$, H–C(10)); 6.25 (*m*, H–C(14,14′)); 6.36 (*d*, $J(12,11) = 14.7$, $J(12',11') = 14.1$, H–C(12,12′)); 6.37 (*d*, $J(8,7) = 16.0$, H–C(8)); 6.62 (*d*, $J(10,11) = 11.4$, $J(11,12) = 14.7$, H–C(11)); 6.63 (*m*, H–C(15,15′)); 6.64 (*dd*, $J(11',10') = 11.3$, $J(11',12') = 14.1$, H–C(11′)). ^{13}C-NMR ($CDCl_3$): 12.75 (C(20′)); 12.80 (C(19)[1])); 12.81 (C(19′)[1])); 12.84 (C(20)[1])); 21.62 (C(18′)); 25.72 (C(16)); 28.72 (C(16′)); 30.25 (C(17′)); 31.58 (C(18));: 32.16 (C(17)); 37.12 (C(1′)); 42.56 (C(4′)); 43.98 (C(1)); 47.71 (C(4)); 48.43 (C(2′)); 48.50 (C(2)); 65.09 (C(3′)); 75.37 (C(3)); 82.48 (C(5)); 91.64 (C(6)); 122.83 (C(7)); 124.81 (C(11)[2])); 124.91 (C(11′)[2])); 125.56 (C(7′)); 126.15 (C(5′)); 130.05 (C(15)[3])); 130.10 (C(15′)[3])); 131.31 (C(10′)); 131.60 (C(10)); 132.58 (C(14)); 132.66 (C(14′)); 134.77 (C(8)); 134.86 (C(9)); 135.40 (C(13)); 136.40 (C(9′)); 136.49 (C(13′)); 137.57 (C(12)); 137.76 (C(12′)); 137.78 (C(6′)); 138.50 (C(8′)). EI-MS: 584 (33, M^+), 566, (3, $[M - H_2O]^+$), 504 (6, $[M$ – methylcyclopentadiene$]^+$), 492 (2, $[M$ – toluene$]^+$), 299 (7), 286 (16), 221 (29), 181 (23), 160 (39), 155 (30), 119 (53), 105 (43), 55 (35), 43 (100).

5. *Cucurbitaxanthin B* (= *(3S,5R,6R,3′S,5′R,6′S)-3,6:5′,6′-Diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol*; **6**): Orange needles. M.p. 175–177°. UV/VIS (benzene): 483, 453, 427; after acid treatment: 460, 432, 407. CD (EPA, r.t.): 228 (+2.13), 267 (−5.77), 327 (+0.94). CD (EPA, −180°): 229 (+3.36), 269 (−8.51), 355 (+1.31), 463 (−0.20), 480 (+0.47), 488 (−0.88). ^{1}H-NMR: ($CDCl_3$): 0.88 (*s*, Me(17)); 0.98 (*s*, Me(16′)); 1.15 (*s*, Me(17′)); 1.19 (*s*, Me(18′)); 1.21 (*s*, Me(18)); 1.24 (*m*, overlapped, H_{ax}–C(2′)); 1.43 (*s*, Me(16)); 1.61 (*d*, $J_{gem} = 11.5$, H_{eq}–C(2)); 1.62 (*m*, $J_{gem} = 14.3$, H_{ax}–C(4′)); 1.63 (*m*, overlapped, H_{eq}–C(2′)); 1.67 (*d*, $J_{gem} = 11.9$, H_{eq}–C(4)); 1.84 (*ddd*, $J_{gem} = 11.5$, $J(2ax,3) = 5.9$, $J(2ax,4ax) = 2.2$, H_{ax}–C(2)); 1.93 (*s*, Me(19′)); 1.95 (*s*, Me(19)); 1.96 (*s*, Me(20,20′)); 2.06 (*ddd*, $J_{gem} = 11.9$, $J(4ax,3) = 6.0$, $J(4ax,2ax) = 2.2$, H_{ax}–C(4)); 2.39 (*ddd*, $J_{gem} = 14.3$, $J(4'eq,3') = 5.2$, $J(4'eq,2'eq) = 1.8$, H_{eq}–C(4′)); 3.91 (*m*, H–C(3′)); 4.39 (‘*t*’, $J(3,2ax) = 5.9$, $J(3,4ax) = 6.0$, H–C(3)); 5.74 (*d*, $J(7,8) = 16.0$, H–C(7)); 5.88 (*d*, $J(7',8') = 15.5$, H–C(7′)); 6.20 (*d*, $J(10',11') \approx 11.5$, H–C(10′)); 6.21 (*d*, $J(10,11) \approx 11.5$, H–C(10)); 6.26 (*m*, H–C(14,14′)); 6.29 (*d*, $J(8',7') = 15.5$, H–C(8′)); 6.37 (*d*, $J(8,7) = 16.0$, H–C(8)); 6.36 (*d*, $J(12,11) = 14.7$, H–C(12)); 6.37 (*d*, $J(12',11') = 14.7$, H–C(12′)); 6.60 (*dd*, $J(11',10') \approx 11.5$, $J(11',12') = 14.7$, H–C(11′)); 6.61 (*dd*, $J(11,10) \approx 11.5$, $J(11,12) = 14.7$, H–C(11)); 6.63 (*m*, H–C(15,15′)). ^{13}C-NMR ($CDCl_3$): 12.9 (C(19,19′), C(20,20′)); 20.1 (C(18′)); 25.1 (C(16′)); 25.9 (C(16)); 29.7 (C(17′)); 31.8 (C(18)); 32.3 (C(17)); 41.3 (C(4′)); 47.9 (C(2′)); 48.2 (C(4)); 48.9 (C(2)); 65.0 (C(3′)); 76.2 (C(3)); 123.0 (C(7)); 123.9 (C(7′)); 124.7 (C(11′)); 124.8 (C(11)); 130.1 (C(15,15′)); 132.2 (C(10,10′)); 132.8 (C(14,14′)); 135.0 (C(8)); 137.6 (C(8′)); 138.3 (C(12,12′)). EI-MS: 600 (50, M^+), 582 (9, $[M - H_2O]^+$), 564 (3, $[M - 2H_2O]^+$), 520 (21, $[M$ – methylcyclopentadiene$]^+$), 508 (21, $[M$ – toluene$]^+$), 287 (36), 286 (32), 221 (100), 181 (57), 160 (45), 155 (26), 119 (32), 105 (26), 55 (10), 43 (25).

6. *Capsanthin 3,6-Epoxide* (= *(3S,5R,6R,3′S,5′R)-3,6-Epoxy-5,6-dihydro-5,3′-dihydroxy-β,κ-caroten-6′-one*; **7**): Red crystals. M.p. 175–177°. UV/VIS (benzene): 507, 481. CD (EPA, r.t.): 243 (−1.34), 281 (+3.38), 353 (−1.31). CD (EPA, −180°): 222 (+0.63), 252 (−3.54), 274 (−0.48), 278 (−1.17), 286 (+1.47), 296 (−1.94), 343 (+1.20), 353 (−0.63), 360 (+0.89), 373 (−2.34), 391 (−1.63), 484 (−2.88), 509 (−1.67), 517 (−2.24). ^{1}H-NMR: ($CDCl_3$): 0.84 (*s*, Me(16′)); 0.88 (*s*, Me(17)); 1.20 (*s*, Me(17′)); 1.21 (*s*, (Me(18)); 1.36 (*s*, Me(18′)); 1.43 (*s*, Me(16)); 1.48 (*dd*, $J_{gem} = 14.5$, $J(4'ax,3') = 3.3$, H_{ax}–C(4′)); 1.61 (*d*, $J_{gem} = 11.6$, H_{eq}–C(2)); 1.67 (*d*, $J_{gem} = 11.9$, H_{eq}–C(4)); 1.71 (*dd*, $J_{gem} = 13.8$, $J(2'ax,3') = 4.7$, H_{ax}–C(2′)); 1.84 (*ddd*, $J_{gem} = 11.6$, $J(2ax,3) = 6.0$, $J(2ax,4ax) = 2.4$, H_{ax}–C(2)); 1.99 (*dd*, $J_{gem} = 13.8$, $J(2'eq,3') = 7.8$, H_{eq}–C(2′)); 1.95 (*s*, Me(19,19′)); 1.97 (*s*, Me(20′)); 1.98 (*s*, Me(20)); 2.06 (*ddd*, $J_{gem} = 11.9$, $J(4ax,3) = 6.0$, $J(4ax,2ax) = 2.4$, H_{ax}–C(4)); 2.95 (*dd*, $J_{gem} = 14.5$, $J(4'eq,3') = 8.5$, H_{eq}–C(4′)); 4.39 (‘*t*’, $J(3,2ax) \approx J(3,4ax) = 6.0$, H–C(3)); 4.51 (*m*, H–C(3′)); 5.75 (*d*, $J(7,8) = 16.1$, H–C(7)); 6.20 (*d*, $J(10,11) = 11.5$, H–C(10)); 6.26 (*m*, H–C(14)); 6.35 (*m*, H–C(14′)); 6.36 (*d*, $J(12,11) = 14.7$, H–C(12)); 6.37 (*d*, $J(8,7) = 16.1$, H–C(8)); 6.44 (*d*, $J(7',8') = 15.2$, H–C(7′)); 6.51 (*d*, $J(12',11') = 14.5$, H–C(12′)); 6.61 (*dd*, $J(11',10') = 11.0$, $J(11',12') = 14.5$, H–C(11′)); 6.62 (*m*, H–C(15)); 6.65 (*dd*, $J(11,10) = 11.5$, $J(11,12) = 14.7$, H–C(11)); 6.69 (*m*, H–C(15′)); 7.33 (*d*, $J(8',7') = 15.2$, H–C(8′)). ^{13}C-NMR ($CDCl_3$): 12.73 (C(20′)); 12.84 (C(20)[1])); 12.86 (C(19,19′)[1])); 21.28 (C(18′)); 25.08 (C(17′)); 25.72 (C(16)); 25.85

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[1])[2])[3]) Assignment may be interchanged.

(C(16′)); 31.57 (C(18)); 32.15 (C(17)); 43.96 (C(1′)); 43.98 (C(1)); 45.30 (C(4′)); 47.71 (C(4)); 48.49 (C(2)); 50.85 (C(2′)); 58.93 (C(5′)); 70.36 (C(3′)); 75.37 (C(3)); 82.47 (C(5)); 91.64 (C(6)); 120.87 (C(7′)); 123.10 (C(7)); 124.08 (C(11′)); 125.40 (C(11)); 129.72 (C(15)); 131.51 (C(15′)); 131.61 (C(10)); 132.43 (C(14)); 133.63 (C(9′)); 134.82 (C(8)); 135.20 (C(9)); 135.23 (C(14′)); 135.92 (C(13)); 137.51 (C(13′)); 137.60 (C(12)); 140.70 (C(10′)); 141.96 (C(12′)); 146.86 (C(8′)); 202.91 (C(6′)). EI-MS: 600 (8, M^+), 582, (2, $[M - H_2O]^+$), 494 (13), 299 (8), 286 (7), 221 (28), 181 (16), 160 (14), 155 (14), 145 (28), 119 (22), 109 (100), 105 (26), 91 (51), 83 (59), 69 (20), 55 (31), 43 (86).

7. *Cycloviolaxanthin* (= *(3S,5R,6R,3′S,5′R,6′R)-3,6:3′,6′-Diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,5′-diol*; **8**): Orange needles crystallized from benzene/hexane. M.p. 145–147°. UV/VIS (benzene): 483, 453, 427. CD (EPA, r.t.): 237 (−0.80), 274 (+0.81), 331 (−1.03), 417 (−1.13), 425 (−0.82), 446 (−1.84), 460 (−0.98), 472 (−1.80). CD (EPA, −180°): 208.5 (−0.25), 214.5 (−0.24), 224.5 (+1.18), 248.5 (−2.87), 262.5 (−2.20), 268 (−1.46), 275.5 (+0.80), 280.5 (−0.03), 284 (+0.39), 335.5 (−0.59), 420 (−0.89), 421.5 (−0.70), 427.5 (−1.24), 441.5 (−0.22), 451 (−2.28), 465.5 (−1.82), 471.5 (+0.29), 483 (−0.87), 487 (−2.54). ^{1}H-NMR ($CDCl_3$): 0.88 (*s*, Me(17,17′)); 1.21 (*s*, Me(18,18′)); 1.43 (*s*, Me(16,16′)); 1.61 (*d*, J_{gem} = 11.5, H_{eq}–C(2,2′)); 1.67 (*d*, J_{gem} = 12.1, H_{eq}–C(4,4′)); 1.84 (*ddd*, J_{gem} = 11.5, J(2ax,3) = 5.9, J(2ax,4ax) = 2.1, H_{ax}–C(2,2′)); 1.95 (*s*, Me(19,19′)); 1.96 (*s*, Me(20,20′)); 2.05 (*ddd*, J_{gem} = 12.0, J(4ax,3) = 6.2, J(4ax,2ax) = 2.2, H_{ax}–C(4,4′)); 4.39 ('*t*', J(3,2ax) = J(3,4ax) = 6.0, H–C(3,3′)); 5.74 (*d*, J(7,8) = 16.0, H–C(7,7′)); 6.20 (*d*, J(10,11) = 11.4, H–C(10,10′)); 6.26 (*m*, H–C(14,14′)); 6.36 (*d*, J(12,11) = 14.9, H–C(12,12′)); 6.37 (*d*, J(8,7) = 15.9, H–C(8,8′)); 6.62 (*m*, H–C(11,11′), H–C(15,15′)). ^{13}C-NMR ($CDCl_3$): 12.81 (C(19,19′)[1]); 12.85 (C(20,20′)[1])); 25.73 (C(17,17′)); 31.60 (C(18,18′)); 32.17 (C(16,16′)); 44.01 (C(1,1′)); 47.75 (C(4,4′)); 48.53 (C(2,2′)); 75.37 (C(3,3′)); 82.50 (C(5,5′)); 91.66 (C(6,6′)); 122.84 (C(7,7′)); 124.83 (C(11,11′)); 130.11 (C(15,15′)); 131.62 (C(10,10′)); 132.64 (C(14,14′)); 134.77 (C(8,8′)); 134.89 (C(9,9′)); 136.43 (C(13,13′)); 137.81 (C(12,12′)). EI-MS: 600 (100, M^+), 582 (17, $[M - H_2O]^+$), 564 (3, $[M - 2H_2O]^+$), 520 (11, $[M$ – methylcyclopentadiene$]^+$), 508 (6, $[M$ – toluene$]^+$), 286 (30), 221 (35), 181 (24), 160 (22), 155 (15).

8. *Cucurbitachrome 1* (= *(3S,5R,6R,3′S,5′R,8′R)-3,6:5′,8′-Diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol*; **9**): UV/VIS (benzene): 460, 432, 408. ^{1}H-NMR: ($CDCl_3$): 0.88 (*s*, Me(17)); 1.17 (*s*, Me(16′)); 1.21 (*s*, Me(18)); 1.33 (*s*, Me(17′)); 1.43 (*s*, Me(16)); 1.51 (*m*, H_{eq}–C(2′)); 1.61 (*d*, J_{gem} = 11.8, H_{eq}–C(2)); 1.62 (*s*, Me(18′)); 1.67 (*d*, J_{gem} = 12.0, H_{eq}–C(4)); 1.72 (*d*, J(19′,8′) = 1.1, Me(19′)); 1.76 (*ddd*, J_{gem} = 14.2, J(2′ax,3′) = 3.6, J(2′ax,4′ax) ≈ 2.5, H_{ax}–C(2′)); 1.84 (*ddd*, J_{gem} = 11.8, J(2ax,3) = 5.8, J(2ax,4ax) = 2.0, H_{ax}–C(2)); 1.99 (*dd*, J_{gem} = 13.6, J(4′eq,3′) = 4.8, H_{eq}–C(4′)); 1.95 (*s*, Me(19)); 1.96 (*s*, Me(20,20′)); 2.06 (*ddd*, J_{gem} = 12.0, J(4ax,3) = 5.9, J(4ax,2ax) = 2.0, H_{ax}–C(4)); 2.12 (*ddd*, J_{gem} = 13.6, J(4′ax,3′) = 3.3, J(4′ax,2′ax) = 2.5, H_{ax}–C(4′)); 4.25 (*m*, H–C(3′)); 4.39 ('*t*', J(3,2ax) = J(3,4ax) = 5.9, H–C(3)); 5.17 (*m*, H–C(8′)); 5.26 (*d*, J(7′,8′) = 0.9, H–C(7′)); 5.74 (*d*, J(7,8) = 16.1, H–C(7)); 6.19 (*d*, J(10′,11′) = 11.2, H–C(10′)); 6.20 (*d*, J(10,11) = 11.3, H–C(10)); 6.25 (*m*, H–C(14,14′)); 6.32 (*d*, J(12,11) = 15.2, H–C(12)); 6.35 (*d*, J(12′,11′) = 14.9, H–C(12′)); 6.37 (*d*, J(8,7) = 16.1, H–C(8)); 6.49 (*dd*, J(11′,10′) = 11.2, J(11′,12′) = 14.9, H–C(11′)); 6.61 (*dd*, J(11,10) = 11.3, J(11,12) = 15.2, H–C(11)); 6.62 (*m*, J(15,14) ≈ 11.0, H–C(15,15′)). EI-MS: 600 (22, M^+), 582 (1, $[M - H_2O]^+$), 520 (11, $[M$ – methylcyclopentadiene$]^+$), 508 (3, $[M$ – toluene$]^+$), 286 (20), 221 (100), 181 (71), 160 (40), 155 (39), 119 (51), 105 (43), 55 (52), 43 (90).

9. *Cucurbitachrome 2* (= *(3S,5R,6R,3′S,5′R,8′S)-3,6:5′,8′-Diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol*; **10**): Yellow crystals. M.p. 182–184°. UV/VIS (benzene): 460, 432, 408. CD (EPA, r.t.): 231 (−1.91), 251 (−3.76), 313 (+1.16), 423 (−2.31), 437 (−1.19), 452 (−2.45). CD (EPA, −180°): 227 (−2.73), 253 (−7.07), 304 (+1.71), 311 (+0.76), 317 (+2.51), 439 (−1.85), 453 (−0.34), 462 (−2.82), 465 (−2.02), 469 (−3.57). ^{1}H-NMR: ($CDCl_3$): 0.88 (*s*, Me(17)); 1.19 (*s*, Me(16′)); 1.21 (*s*, Me(18)); 1.34 (*s*, Me(17′)); 1.43 (*s*, Me(16)); 1.47 (*dd*, J_{gem} = 14.3, J(2′eq,3′) = 3.8, H_{eq}–C(2′)); 1.61 (*d*, J_{gem} = 11.5, H_{eq}–C(2)); 1.67 (*d*, J_{gem} = 12.1, H_{eq}–C(4)); 1.68 (*s*, Me(18′)); 1.80 (*s*, Me(19′)); 1.80 (*ddd*, J_{gem} = 14.3, J(2′ax,3′) = 3.7, J(2′ax,4′ax) = 1.6, H_{ax}–C(2′)); 1.84 (*ddd*, J_{gem} = 11.5, J(2ax,3) = 5.8, J(2ax,4ax) = 2.0, H_{ax}–C(2)); 1.90 (*dd*, J_{gem} = 13.7, J(4′eq,3′) = 4.7, H_{eq}–C(4′)); 1.95 (*s*, Me(19), Me(20′)); 1.96 (*s*, Me(20)); 2.06 (*ddd*, J_{gem} = 12.1, J(4ax,3) = 6.1, J(4ax,2ax) = 2.0, H_{ax}–C(4)); 2.11 (*ddd*, J_{gem} = 13.7, J(4′ax,3′) = 3.3, J(4′ax,2′ax) = 1.6, H_{ax}–C(4′)); 4.24 (*m*, H–C(3′)); 4.39 (*t*, J(3,2ax) = 5.8, J(3,4ax) = 6.1, H–C(3)); 5.07 (*s*, H–C(8′)); 5.30 (*d*, J(7′,8′) = 1.9, H–C(7′)); 5.73 (*d*, J(7,8) = 15.9, H–C(7)); 6.19 (*d*, J(10′,11′) = 10.7, H–C(10′)); 6.20 (*d*, J(10,11) ≈ 11.3, H–C(10)); 6.22 H–C(14′)); 6.25 (H–C(14)); 6.32 (*d*, J(12′,11′) = 15.4, H–C(12′)); 6.35 (*d*, J(12,11) = 14.1, H–C(12)); 6.37 (*d*, J(8,7) = 15.91, H–C(8)); 6.50 (*dd*, J(11′,10′) = 10.7, J(11′,12′) = 15.4, H–C(11′)); 6.61 (*dd*, J(11,10) ≈ 11.3, J(11,12) ≈ 14.1, H–C(11)); 6.63 (H–C(15,15′)). ^{13}C-NMR ($CDCl_3$): 12.80 (C(20,20′)[1])); 12.84 (C(19)[1])); 13.40 (C(19′)); 25.72 (C(17)); 28.17 (C(17′)); 30.56 (C(18′)); 31.26 (C(16′)); 31.59 (C(18)); 32.16 (C(16)); 34.22 (C(1′)); 43.98 (C(1)); 47.38 (C(2′)); 47.40 (C(4′)); 47.71 (C(4)); 48.51 (C(2)); 67.94 (C(3′)); 75.36 (C(3)); 82.49 (C(5)); 87.17 (C(5′)); 88.38 (C(8′)); 91.63 (C(6)); 118.74 (C(7′)); 122.78 (C(7)); 124.50 (C(11′)); 124.73 (C(11)); 126.18 (C(10′)); 129.84 (C(15′)[2])); 130.08 (C(15)[2])); 131.60 (C(10)); 132.15 (C(14′)); 132.63 (C(14)); 134.24 (C(13′)[3])); 134.34 (C(13)[3])); 134.70 (C(9)); 134.87

(C(8)); 137.36 (C(12')); 137.81 (C(12)); 138.65 (C(9')); 153.19 (C(6')). EI-MS: 600 (68, M^+), 582 (3, $[M - H_2O]^+$), 520 (31, $[M$ – methylcyclopentadiene$]^+$), 508 (13, $[M$ – toluene$]^+$), 286 (31), 221 (100), 181 (41), 160 (48), 155 (25), 119 (22), 105 (19), 55 (88), 43 (19).

10. *3,6-Epoxy-5,6,5',6'-tetrahydro-β,β-carotene-5,3',5',6'-tetrol* (**11**): UV/VIS (benzene): 483, 453, 427; no furanoid reaction. CD (EPA, r.t.): 235 (−0.35), 264 (+0.432), 472 (−1.14). EI-MS: 618 (15, M^+), 600 (4, $[M - H_2O]^+$), 582 (3, $[M - 2H_2O]^+$), 538 (2, $[M$ – methylcyclopentadiene$]^+$), 286 (10), 221 (33), 181 (26), 160 (22), 155 (15), 119 (21), 105 (18), 55 (31), 43 (100).

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