# Confirmation of the Absolute (3*R*,3′*S*,6′*R*)-Configuration of (all-*E*)-3′-Epilutein

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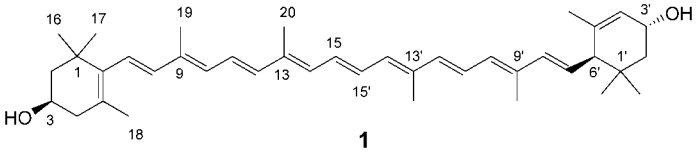
Circular dichroism (CD) spectroscopy was used to distinguish between the isomeric (all-*E*)-configured 3′-epilutein (**2**) and 6′-epilutein (**8**) to establish the absolute configuration of epilutein samples of different (natural and semisynthetic) origin, including samples of **2** obtained from thermally processed sorrel. Thus, the CD data of lutein (**1**) and epilutein samples (**2**) were compared. Our results unambiguously confirmed the (3*R*,3′*S*,6′*R*)-configuration of all epilutein samples. Compound **2** was thoroughly characterized, and its $^{13}$C-NMR data are published herewith for the first time.

**Introduction**. – Lutein (=(all-*E*,3*R*,3′*R*,6′*R*)-4′,5′-didehydro-5′,6′-dihydro-β,β-carotene-3,3′-diol; **1**)) is the main xanthophyll found in the major light-harvesting pigment–protein complex of higher plants, and is involved in energy-transfer mechanisms during photosynthesis. In the fatty acid ester form, **1** is widely distributed in fruits, flowers, and yellow autumn leaves [1]. The 3′-epimer of lutein, *i.e.*, 3′-epilutein (**2**), with the (all-*E*,3*R*,3′*S*,6′*R*)-configuration, was isolated from the flowers of Marsh Marigold (*Caltha palustris*) [2][3] and from goldfish (*Carassius auratus*), together with α-doradexanthin (=(all-*E*,3*S*,3′*S*,6′*R*)-3,3′-dihydroxy-4′,5′-didehydro-5′,6′-dihydro-β,β-caroten-4-one; **3**) [4]. Compound **2** was also detected in the anthers of flowers such as *Rosa gallica* '*officinalis*' THORY and *Paeonia officinalis* [5]. It was later established that **1** and **2** are widespread also in the animal world [6].

Lutein (**1**), 3′-epilutein (**2**), zeaxanthin (=(3*R*,3′*R*)-β,β-carotene-3,3′-diol; **4**) and several (*Z*)-isomers thereof, 3′-oxolutein (=(3*R*,6′*R*)-3-hydroxy-4′,5′-didehydro-5′,6′-dihydro-β,β-caroten-3′-one; **5**), even-numbered dehydration products of lutein, namely anhydrolutein I (=(all-*E*,3*R*,6′*R*)-3′,4′,5′,18′-tetradehydro-5′,6′-dihydro-β,β-caroten-3-ol; **6**) and anhydrolutein II (=(all-*E*,3*R*,6′*S*)-2′,3′,4′,5′-tetradehydro-5′,6′-dihydro-β,β-caroten-3-ol; **7**), as well as a number of other carotenoids and their oxidation products, have been identified in the extracts of human plasma [7–9]. In the last few years, compounds **1**, **2**, **4**, and **5**, together with mesozeaxanthin and several (*Z*)-isomers of **1** and **4**, have also been detected and identified in human and monkey retina [10][11], and in human eye tissues [12][13]. It was established that lutein (**1**), zeaxanthin (**4**), their (*Z*)-isomers, and their metabolites, namely **2** and **5**, play an important role in the prevention of age-related macular degeneration (AMD) [12]. In connection with these investigations, detailed photochemical studies of 3′-oxolutein (**5**) have been reported [14].


16
17
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3
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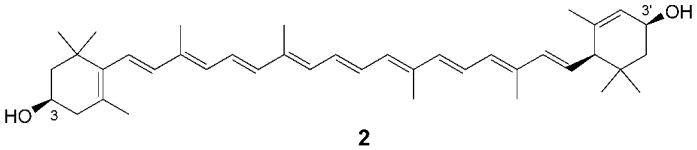
**1**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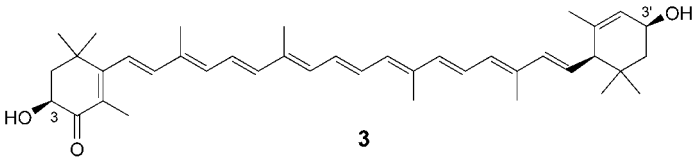
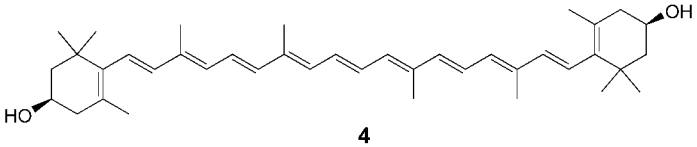
OH
HO
3

**2**


3'
OH
HO
3
O

**3**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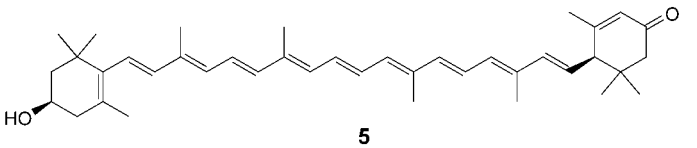
**4**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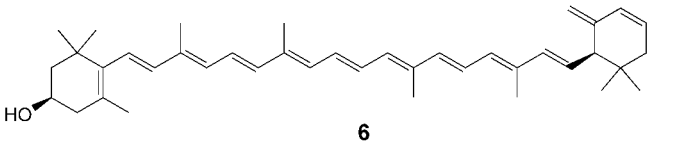
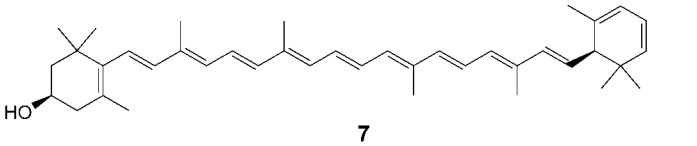
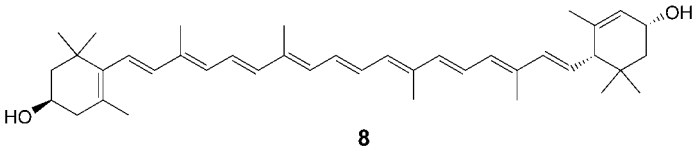
**5**


HO

**6**


HO

**7**


OH
HO

**8**

3′-Epilutein (**2**) and 3′-oxolutein (**5**) have been prepared from lutein (**1**) and characterized by UV/VIS-, IR-, $^1$H-NMR-, CD, and mass spectroscopy [2–4][15–18]. Considering the important role of **2**, novel industrial processes have been developed recently for the production of this carotenoid [19][20].

We have reported [21] that lutein (**1**), the main xanthophyll of many fruits and vegetables, can be converted by heating in acidic medium to an epilutein (3′- or 6′-stereoisomers **2** or **8**, resp.) and to anhydrolutein I (**6**), which, to the best of our knowledge, is the first observation of such an epimerization of lutein in natural samples.

Since NMR methods are not suited to distinguish between the epiluteins **2** and **8**, and to establish the absolute configuration at C(3′) and C(6′) of **1** isolated from processed sorrel (*Rumex rugisus camp.*), we turned to circular dichroism (CD) spectroscopy and compared the CD data of **1** with those of epilutein samples of different origin: *a*) isolated from the extract of the flowers of *Caltha palustris*, *b*) prepared from 3′-oxolutein (**5**) by $NaBH_4$-reduction, and *c*) obtained from lutein (**1**) by acid-catalyzed epimerization.

**Results.** – *Isolation of 3′-Epilutein* (**2**) *from Processed Sorrel*. The main carotenoids of fresh sorrel are lutein and $\beta$-carotene, and according to its acid content, some furanoids (neochromes, mutatoxanthins) were also observed. Applying different thermal methods, the formation of 3′-epilutein (7–12%) as well as of anhydrolutein I (3–7%) could be detected by HPLC [21]. Starting from 500 g of steamed (10 min) sorrel, after extraction and repeated column chromatography (see *Exper. Part*), a total of 3 and 1 mg of cristalline lutein (**1**) and 3′-epilutein (**2**) were isolated, respectively.

*Isolation of 3′-Epilutein* (**2**) *from the Flowers of* Caltha palustris. The HPLC separation of the hypophasic carotenoids of the extracts of *Caltha palustris* is shown in *Fig. 1*. The main carotenoids isolated were again **1** and **2**. As minor compounds, neoxanthin, (9*Z*)-neoxanthin, violaxanthin, a luteoxanthin epimer, (*Z*)-luteoxanthins, (9*Z*)-violaxanthin, antheraxanthin, $\alpha$-cryptoxanthin, $\beta$-carotene, as well as (*Z*)-isomers of both **1** and **2** were detected. In accordance with the investigations of *Eugster* and co-workers [3], we isolated **1** and **2** in highly pure (> 95%) crystalline form by preparative column chromatography (see *Exper. Part*).

*Semisynthetic Preparation of 3′-Epilutein* (**2**). Compound **2** was prepared by $NaBH_4$ reduction [3] of 3′-oxolutein (**5**; 6 mg), prepared according to [22], in benzene/EtOH 1:1, resulting in the formation of of lutein (**1**; 2 mg) and 3′-epilutein (**2**; 1.8 mg) after separation by column chromatography (see *Fig. 2*, *a*).

Compound **2** was also prepared by acid-catalyzed epimerization of **1** (50 mg) in THF/$H_2O$ 1:1 in the presence of aqueous HCl, which resulted in a mixture containing **1** and **2** as the main products, together with the following side products: (9*Z*)-**1**, (9′*Z*)-**1**, (13′*Z*)-**1**, (13′*Z*)-**1**, and (15*Z*)-**1**) [23]; different (*Z*)-isomers of **2** [24]; anhydrolutein I (**6**) [13][25][26] as well as the (9*Z*,9′*Z*)- and (13*Z*,13′*Z*)-isomers of **6** (*Fig. 2*, *b*). Thereby, the (*Z*)-isomers of both **1** and **2**, as well as compound **6**, were identified by co-chromatography with authentic samples [23–25], and the (*Z*)-isomers of **6** were identified by UV/VIS spectroscopy. After HPLC separation (*Fig. 2*, *b*) of the epimerization mixture and recrystallization, 3′-epilutein (**2**; 5.1 mg) was obtained in pure form.

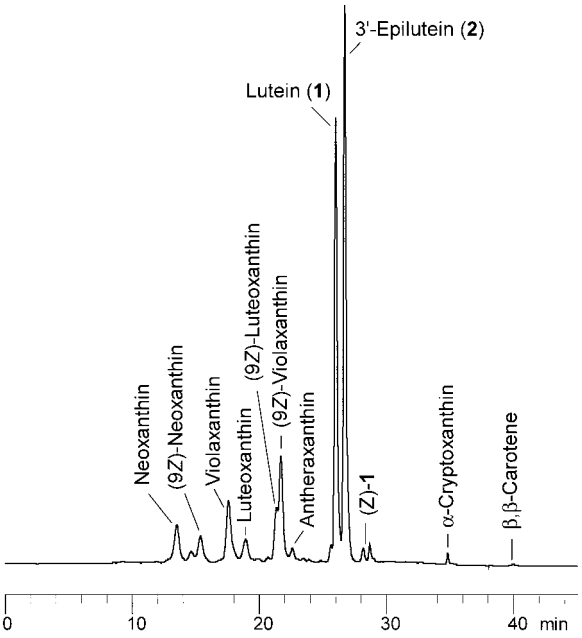



Fig. 1. *HPLC Chromatogram of hypophasic carotenoids isolated from the flowers of* Caltha palustris

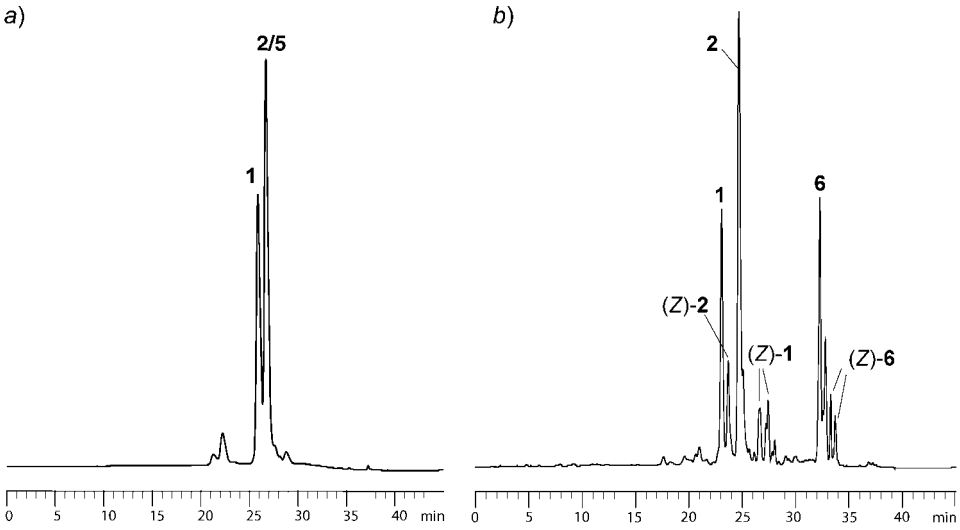



Fig. 2. *a*) *HPLC Profile of the reaction mixtures obtained by* a) *$NaBH_4$ reduction of 3′-oxolutein* (**5**) *or* b) *acid-catalyzed epimerization of lutein* (**1**)

*Spectroscopic Characterization*. The structure elucidation of the above epilutein samples obtained from different sources was carried out by UV/VIS, NMR, CD, and

MS methods. The UV/VIS data ($\lambda_{max}$ 488, 457, and 434 nm; in benzene) corresponded well with the reported values for (all-*E*)-lutein (**1**), and are characteristic for carotenoids with $\beta$- and $\varepsilon$-type end groups on the nonaene polyene chain.

The mass spectral data of all epilutein samples exhibited the same pattern, with the molecular-ion peak at *m/z* 568 ($M^+$, $C_{40}H_{56}O_2^+$), and fragments at *m/z* 550 ($[M-H_2O]^+$), 476 ($[M-C_7H_8]^+$), 235, 223, 209, 173, 157, 145, 119, 95, 69, and 43.

The assignment of $^1$H- and $^{13}$C-NMR signals were corroborated by $^1$H,$^1$H-COSY, gradient-enhanced $^{13}$C,$^1$H-HSQC, and $^{13}$C,$^1$H-HMBC experiments performed with the standard *Varian* software. The difference in the spatial arrangement of the H-atoms at C(3′) and C(6′) in **1** *vs.* **2** (on opposite and on the same side of the cyclohexene ring, respectively), was further confirmed by TROESY experiments. The $^1$H-NMR data ($\delta$(H) and *J*(H,H) values) were found to be identical for all isolated and semisynthetic epilutein samples, and were in accordance with the literature data [27–30]. However, according to the literature data of the eight theoretically possible (all-*E*)-lutein stereoisomers (lutein A–H), the characteristic $^1$H-NMR signals were found to be the same for lutein stereoisomers possessing a (3′,6′-*cis*)-configured $\varepsilon$-type end group (lutein B, F, G, and H), but different for stereoisomers with a (3′,6′-*trans*)-configured $\varepsilon$-type end group (lutein A, C, D, E) [27–30]. Hence, the different NMR methods were not suited to distinguish between the 3′- and 6′-epilutein isomers, 3′-epilutein (= lutein B; **2**) having the (3′*S*,6′*R*)-, and 6′-epilutein (= lutein F; **8**) the (3′*R*,6′*S*)-configuration, respectively.

In the *Table* below, the complete $^1$H- and $^{13}$C-NMR assignments are given for both lutein (**1**) and 3′-epilutein (**2**) isolated from the flowers of *Caltha palustris*. We found that the chemical shifts of the characteristic $\varepsilon$-type end groups ($H_\alpha$–C(2′), $H_\beta$–C(2′), H–C(3′) of different epilutein samples were absolutely identical.

To establish the absolute configurations at C(3′) and C(6′), the CD-spectroscopic data of the samples of lutein (**1**) and 3′-epilutein (**2**) of different origins were compared. For $\varepsilon$-ring carotenoids, the CD spectra are generally nonconservative [31]. Substitution at C(3′) has no influence on the sign of the *Cotton* effect, nor on the general shape of the spectrum. In our case, differences were observed only in terms of shifts in maximum and minimum wavelengths, as determined by the comparison of the CD spectrum of **1** with that of **2** isolated from the flowers of *Caltha palustris* (*Fig. 3*). The absolute configuration at C(3′) of **1** and **2** could, thus, not be deduced from their CD spectra [31].

Both lutein (**1**) and 3′-epilutein (**2**) possess on one side optically active $\beta$-type and, on the other, optically active $\varepsilon$-type end groups. Since the $\beta$-type end group is conjugated with the polyene chain, the absolute configuration of C(3) determines the helicity of the chromophore *via* the steric influence of the asymmetric terminal ring on the dihedral angle about the C(6)–C(7) bond. In contrast, 3′- and 6′-centers of the unconjugated $\varepsilon$-type end groups, showing high degrees of conformational freedom, exert only a weak chiral perturbation on the electronic transitions of the conjugated chain.

The CD spectrum of epilutein isolated from *Caltha palustris* was very similar to those of all other investigated epilutein samples (*Fig. 4*), but these spectra were different from the CD spectrum of 6′-epilutein (**8**) isolated from marine fish [6][27]

Table. *$^1$H- and $^{13}$C-NMR Data of 3′-Epilutein* (**2**) *and Lutein* (**1**) *Isolated from the Flowers of* Caltha palustris. Conditions: at 400 and 100 MHz, resp., in $CDCl_3$ solution ($T = 25°$); $\delta$ in ppm, $J$ in Hz.

| Position | **2** | | **1** | |
|---|---|---|---|---|
| | $\delta$(H) ($J$) | $\delta$(C) | $\delta$(H) ($J$) | $\delta$(C) |
| 1 | – | 37.1 | – | 37.1 |
| 2 | 1.76 (*ddd*, $J(2\alpha,3) = 3.4$, $J(2\alpha,4\alpha) = 2.0$), 1.47 (*t*-like, $^2J = 11.9$, $J(2\beta,3) = 11.9$) | 48.4 | 1.77 (*ddd*, $J(2\alpha,3) = 3.4$, $J(2\alpha,4\alpha) = 2.1$), 1.47 (*t*-like, $^2J = 11.9$, $J(2\beta,3) = 11.9$) | 48.4 |
| 3 | 3.99 (*m*) | 65.1 | 3.99 (*m*) | 65.9 |
| 4 | 2.38 (*dd*, $J(4\alpha,3) = 5.6$) 2.03 (*dd*, $^2J = 16.9$, $J(4\beta,3) = 9.5$) | 42.6 | 2.38 (*dd*, $J(4\alpha,3) = 5.7$) 2.04 (*dd*, $^2J = 16.8$, $J(4\beta,3) = 9.5$) | 42.5 |
| 5 | – | 126.2 | – | 126.2 |
| 6 | – | 137.7 | – | 138.0 |
| 7 | 6.09 (*m*, $J(7,8) = 16.3$) | 125.6 | 6.09 (*m*, $J(7,8) = 16.3$) | 124.9 |
| 8 | 6.12 (*m*) | 138.5 | 6.12 (*m*) | 138.5 |
| 9 | – | 135.7 | – | 135.7 |
| 10 | 6.14 (*d*, $J(10,11) = 11$) | 131.3 | 6.15 (*m*) | 131.3 |
| 11 | 6.64 (*m*) | 124.9 | 6.64 (*m*) | 124.8 |
| 12 | 6.35 (*d*, $J(11,12) = 14.9$) | 137.6 | 6.35 (*d*, $J(11,12) = 14.8$) | 137.5 |
| 13 | – | 136.5 | – | 136.5 |
| 14 | 6.25 (*m*) | 132.6 | 6.26 (*m*) | 132.6 |
| 15 | 6.62 (*m*) | 130.1 | 6.62 (*m*) | 130.1 |
| 16 | 1.06 (*s*) | 28.7[a]) | 1.07 (*s*) | 28.7 |
| 17 | 1.06 (*s*) | 30.2[a]) | 1.07 (*s*) | 30.2 |
| 18 | 1.73 (*s*) | 21.6 | 1.73 (*s*) | 21.6 |
| 19 | 1.96 (*s*) | 12.8[b]) | 1.97 (*s*) | 12.7 |
| 20 | 1.95 (*s*) | 12.7[b]) | 1.96 (*s*) | 12.8 |
| 1′ | – | 34.8 | – | 34.0 |
| 2′ | 1.62[c]), 1.38 (*dd*, $^2J = 12.6$, $J(2'\beta,3') = 9.6$) | 41.0 | 1.84 (*dd*), 1.37 (*dd*, $^2J = 12.9$, $J(2'\beta,3') = 6.7$) | 44.6 |
| 3′ | 4.22 (*m*) | 66.8 | 4.24 (*m*) | 65.1 |
| 4′ | 5.47 (br. *s*) | 124.4 | 5.54 (br. *s*) | 125.6 |
| 5′ | – | 138.1 | – | 137.7 |
| 6′ | 2.15 (*d*, $J(6',7') = 9.3$) | 55.1 | 2.40 (*d*, $J(6',7') = 10.1$) | 54.9 |
| 7′ | 5.52 (*dd*, $J(7',8') = 15.3$) | 129.8 | 5.42 (*dd*, $J(7',8') = 15.5$) | 128.7 |
| 8′ | *ca.* 6.12 (*m*) | 136.7 | 6.13 (*m*) | 137.7 |
| 9′ | – | 135.3 | – | 135.1 |
| 10′ | *ca.* 6.12 (*m*) | 130.8 | 6.14 (*m*) | 130.8 |
| 11′ | 6.57 (*m*) | 124.3 | 6.60 (*dd*, $J(10',11') = 11.4$ | 124.5 |
| 12′ | 6.34 (*d*, $J(11',12') = 14.9$) | 137.5 | 6.35 (*d*, $J(11',12') = 14.8$) | 137.5 |
| 13′ | – | 136.4 | – | 136.4 |
| 14′ | 6.23 (*m*) | 132.5 | 6.24 (*m*) | 132.6 |
| 15′ | 6.62 (*m*) | 130.0 | 6.62 (*m*) | 130.0 |
| 16′ | 0.84 (*s*) | 27.0 | 0.84 (*s*) | 29.5 |
| 17’ | 0.93 (*s*) | 29.3 | 0.99 (*s*) | 24.3 |
| 18′ | 1.63 (*s*) | 22.6 | 1.61 (*s*) | 22.9 |
| 19′ | 1.90 (*s*) | 13.1[b]) | 1.90 (*s*) | 13.1 |
| 20’ | 1.95 (*s*) | 12.8[b]) | 1.96 (*s*) | 12.8 |

[a]), [b]) Assignments may be interchanged. [c]) Signal overlapped.

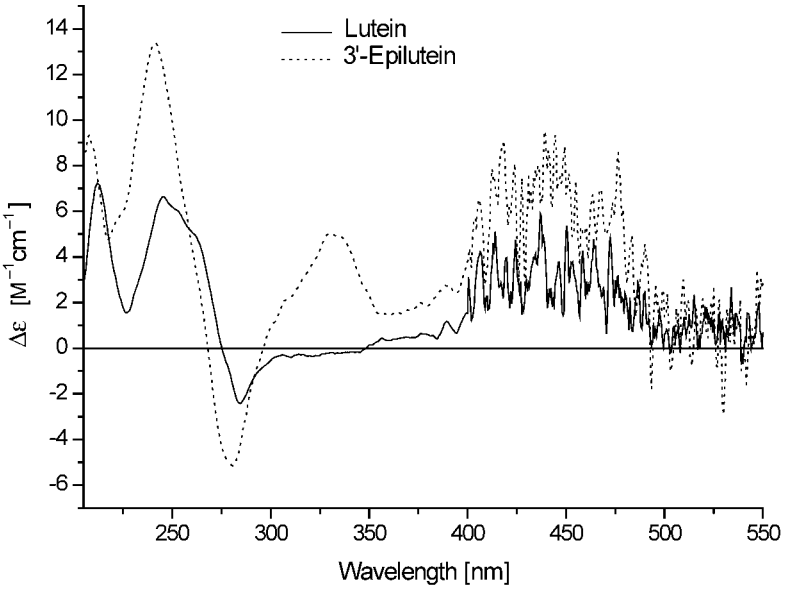



Fig. 3. *CD Spectra of lutein* (**1**) *and 3′-epilutein* (**2**) *isolated from the flowers of* Caltha palustris. Recorded in EtOH at ambient temperature.

(see Fig. 3 and Fig. 5 in [6]). This observation indicates the presence of a (3′,6′-*cis*)-configured ε-type end group with the (3′*S*,6′*R*)-configuration in our epilutein samples.

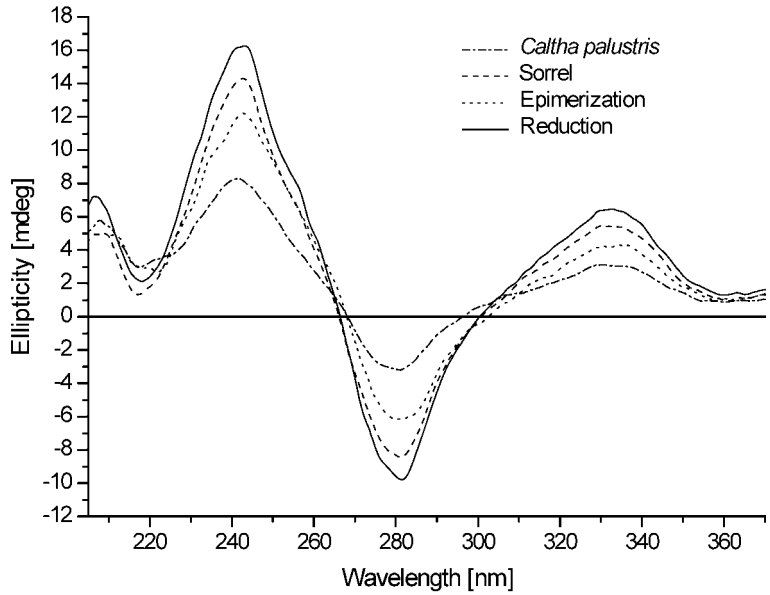



Fig. 4. *Overlay of CD spectra* (in EtOH) *of 3′-epilutein samples of different origins.* Note: the differential signal intensities are due to variations in sample concentration.

**Discussion.** – We could not find any difference between the CD spectra of 3′-epilutein (**2**), obtained by reduction of **5**, and the CD spectra of epilutein samples obtained by epimerization, or directly isolated from either *Caltha palustris* or thermally treated sorrel. since the absolute configuration at C(6′) remained unchanged during the reduction of **5**, the above observation unequivocally corroborated the (3′*S*,6′*R*)-configuration of epilutein samples of different origins.

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## Experimental Part

1. *General*. Column chromatography (CC) was performed over $CaCO_3$ (*Biogal*, Hungary) with columns of 5 × 30 cm or 6 × 30 cm in size. HPLC: *Dionex-580* pump, *HP-1050* detector with *HP* ChemStation software and *Waters 991* photodiode-array detector, *Chromsyl* $C_{18}$ (6-μm endcapped) column (250 × 4.6 mm i.d.); gradient elution (in linear steps) with solvent *A* ($H_2O$/MeOH 12:78), *B* (MeOH), and *C* ($CH_2Cl_2$/MeOH 30:70): 0–2 min 100% *A*; 2–10 min to *A*/*B* 80:20; 10–18 min to *A*/*B* 50:50; 18–25 min to 100% *B*; 25–27 min 100% *B*; 27–34 min to 100% C; 34–41 min 100% *C*, at a flow rate of 1.25 ml/min. UV/VIS: *Jasco V-530* spectrophotometer; $\lambda_{max}$ in nm. CD: *Jasco J-715* spectropolarimeter, $\lambda$ in nm ($\Delta\varepsilon$ in $M^{-1}\,cm^{-1}$); in EtOH at r.t. NMR Spectra: *Varian Unity Inova 400-WB* spectrometer; at 400 ($^1H$) and 100 MHz ($^{13}C$), resp.; in $CDCl_3$ soln. at 25°; chemical shifts $\delta$ in ppm rel. to $Me_4Si$ ($^1H$) or to residual solvent signals ($^{13}C$). MS: *Varian MAT-CH-7A* mass spectrometer; in *m*/*z* (rel%).

2. *Isolation of 3′-Epilutein* (**2**) *from Steamed Sorrel.* Fresh sorrel leaves (500 g), steamed for 20 min, were used for extraction. The material was blended with MeOH in the presence *ca*. 1% $CaCO_3$. The blend was allowed to stand in MeOH for dehydration. After 20 h, the mixture was filtered, and the filter cake was extracted with MeOH (2×) and $Et_2O$. The MeOH and $Et_2O$ extracts were combined, diluted with $Et_2O$, washed with $H_2O$ to remove MeOH, dried ($Na_2SO_4$), concentrated under vacuum to *ca*. half volume, and saponified with 30% aq. KOH/MeOH at r.t. for 18 h. After saponification, the etheral soln. was washed free from alkali and evaporated. The resulting residue was dissolved in benzene/hexane and separated by CC ($CaCO_3$; hexane/benzene 3:2). The following fractions were obtained: *Fr. 1*, mixture of neochromes and (*Z*)-isomers of **1**; *Fr. 2*, compound **2**; *Fr. 3*, compound **1**; *Fr. 4*, mixture of **6** and (*Z*)-isomers of **2**; *Fr 5*, β-carotene [32]. *Fr. 2* and *3* were rechromatographed ($CaCO_3$) and crystallized from benzene/hexane 1:5 to give 3 and 1 mg of **1** and **2**, resp.

3. *Isolation of 3′-Epilutein* (**2**) *from* Caltha palustris. In accordance with [3], 200 mg of a mixture of hypophasic carotenoids, previously isolated in our laboratory from the flowers of Marsh Marigold (*Caltha palustris*) [33], were separated by repeated (10×) CC ($CaCO_3$; benzene/hexane 1:1). The following fractions were obtained in order of decreasing affinity: *Fr. 1*, mixture of (9*Z*)-neoxanthin, (9*Z*)-violaxanthin, (*Z*)-isomers of luteoxanthin, and (all-*E*)-luteoxanthin; *Fr. 2*, mixture of (all-*E*)-neoxanthin, (all-*E*)-violaxanthin, and (*Z*)-isomers of **1**; *Fr. 3*, compound **2**; *Fr. 4*, small amount of antheraxanthin; *Fr. 5*, compound **1** [32]. *Fr. 3* and *4* were crystallized from benzene/hexane 1:5 to give 45 mg and 50 mg of **2** and **1**, resp.

4. *Preparation of 3′-Epilutein* (**2**) *by Hydride Reduction.* The $NaBH_4$ reduction of 3′-oxolutein (**5**; 6 mg) was carried out according to [3]. The reaction mixture was separated by CC ($CaCO_3$; benzene/hexane 1:1). The following fractions were obtained (in order of decreasing affinity) [32]: *Fr. 1*, compound **2**; *Fr. 2*, compound **5**; *Fr. 3*, compound **1**. The crystallization of the fractions from benzene/hexane 1:5 resulted in 1.8, 0.8, and 2.0 mg of **2**, **5**, and **1**, respectively.

5. *Preparation of 3′-Epilutein* (**2**) *from Lutein* (**1**) *by Acid-Catalyzed Epimerization*. A soln. of **1** (50 mg) in THF/$H_2O$ 1:1 (50 ml) was epimerized with 0.2% aq. HCl soln. (50 ml) at r.t. during 43 h under $N_2$ in the dark. The reaction was monitored by UV/VIS spectroscopy and HPLC (see *Fig. 2*, *b*). After the usual workup [19][32], the mixture was separated by CC ($CaCO_3$; benzene/hexane 2:3; 6 × 30 cm). The following fractions were obtained: *Fr. 1*, mixture of (*Z*)-isomers, mainly (13*Z*)- and (13′*Z*)-**1**) of lutein [23]; *Fr. 2*, mixture of (9*Z*)- and (9′*Z*)-**1** [23], together with **2** as main component; *Fr. 3*, (*Z*)-isomers of **2** [24]; *Fr. 4*, mixture of **1** (main component) and zeaxanthin (**4**) [19]; *Fr. 5*, mixture of **6** (main component [13][21][25][26]) and (13*Z*/13′*Z*)-

isomer of **6**. *Fr. 2*, *4*, and *5* were rechromatographed ($CaCO_3$) to obtain the corresponding main components. Repeated (2×) CC of *Fr. 2* ($CaCO_3$; acetone/hexane 4:96) resulted in *Fr. 21* ((9*Z*)-**1** and (9′*Z*)-**1** [23]), *Fr. 22* (**2**; 5.1 mg after recrystallization from benzene/hexane 1:5), and *Fr. 23* ((*Z*)-isomers of **2**) [24]. *Fr. 4* was resubmitted (2×) to CC ($CaCO_3$; benzene/hexane 2:3; 5 × 30 cm), and the following two fractions were obtained: *Fr. 41*, compound **1** (4.8 mg after recrystallization from benzene/hexane 1:5); and *Fr. 42*, compound **4** [19]. Repeated CC (2×) of *Fr. 5* (benzene/hexane 5:95 to 10:90) resulted in *Fr. 51* (**6**) and *Fr. 52* ((13*Z*)-**6** and/or (13′*Z*)-**6**) [13][21][25][26].

6. *Analytical Data of 3′-Epilutein* (**2**). 6.1. *Sample Isolated from Sorrel*. M.p. 152–154°. UV/VIS (benzene): 487, 457, 433. UV/VIS (EtOH): 475.5, 447.0, 423.5. CD (EtOH, r.t.): 209 (+3.8), 217 (+1.0), 242.5 (+10.8), 266 (0), 281 (−6.4), 301 (0), 331 (+4.1). $^1$H- and $^{13}$C-NMR: see the *Table*. EI-MS: 568 (100, $M^+$), 550 (9), 476 (3), 235 (4), 223 (6), 209 (9), 197 (11), 173 (12), 157 (18), 145 (27), 134 (16), 119 (17), 105 (14), 95 (10), 81 (7), 69 (6), 55 (6), 43 (10).

6.2. *Sample Isolated from* Caltha palustris. M.p. 156–158°. UV/VIS (benzene): 487, 457, 433. UV/VIS (EtOH): 475, 446, 423. CD (EtOH, r.t.): 208 (+9.3), 217.5 (+4.8), 241.5 (+13.4), 268 (0), 281 (−5.1), 296.5 (0), 330.5 (+5). $^1$H- and $^{13}$C-NMR: see the *Table*. EI-MS: 568 (100, $M^+$), 550 (16), 476 (4), 422 (1), 235 (3), 223 (4), 209 (7), 197 (6), 173 (6), 157 (9), 145 (11), 134 (6), 119 (11), 105 (6), 95 (6), 81 (4), 69 (4), 55 (3), 43 (2).

6.3. *Sample Prepared by Reduction*. M.p. 153–155°. UV/VIS (benzene): 487, 457, 433. UV/VIS (EtOH): 476, 446.7, 423.5. CD (EtOH, r.t.): 207 (+5.0), 218.5 (+1.5), 242.5 (+11.4), 266.5 (0), 281.5 (−6.8), 300 (0), 331 (+4.5). $^1$H- and $^{13}$C-NMR: see the *Table*. EI-MS: 568 (100, $M^+$), 550 (7), 476 (7), 420 (1), 235 (4), 223 (11), 209 (15), 197 (13), 173 (14), 157 (21), 145 (32), 134 (22), 119 (35), 105 (26), 95 (23), 81 (14), 69 (12), 55 (15), 43 (16).

6.4. *Sample Prepared by Epimerization*. M.p. 148–150°. UV/VIS (benzene): 487, 457, 433. UV/VIS (EtOH): 475, 446, 423. CD (EtOH, r.t.): 242.5 (+9.6), 268 (0), 280 (−4.8), 302.5 (0), 334.5 (+3.4). $^1$H- and 13C-NMR: see the *Table*. EI-MS: 568 (100, $M^+$), 550 (11), 476 (5), 422 (2), 235 (5), 223 (8), 209 (11), 197 (10), 173 (10), 157 (15), 145 (22), 134 (14), 119 (23), 105 (16), 95 (14), 81 (9), 69 (8), 55 (9), 43 (8).

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