(*E*/*Z*)-Isomerization of 3′-Epilutein

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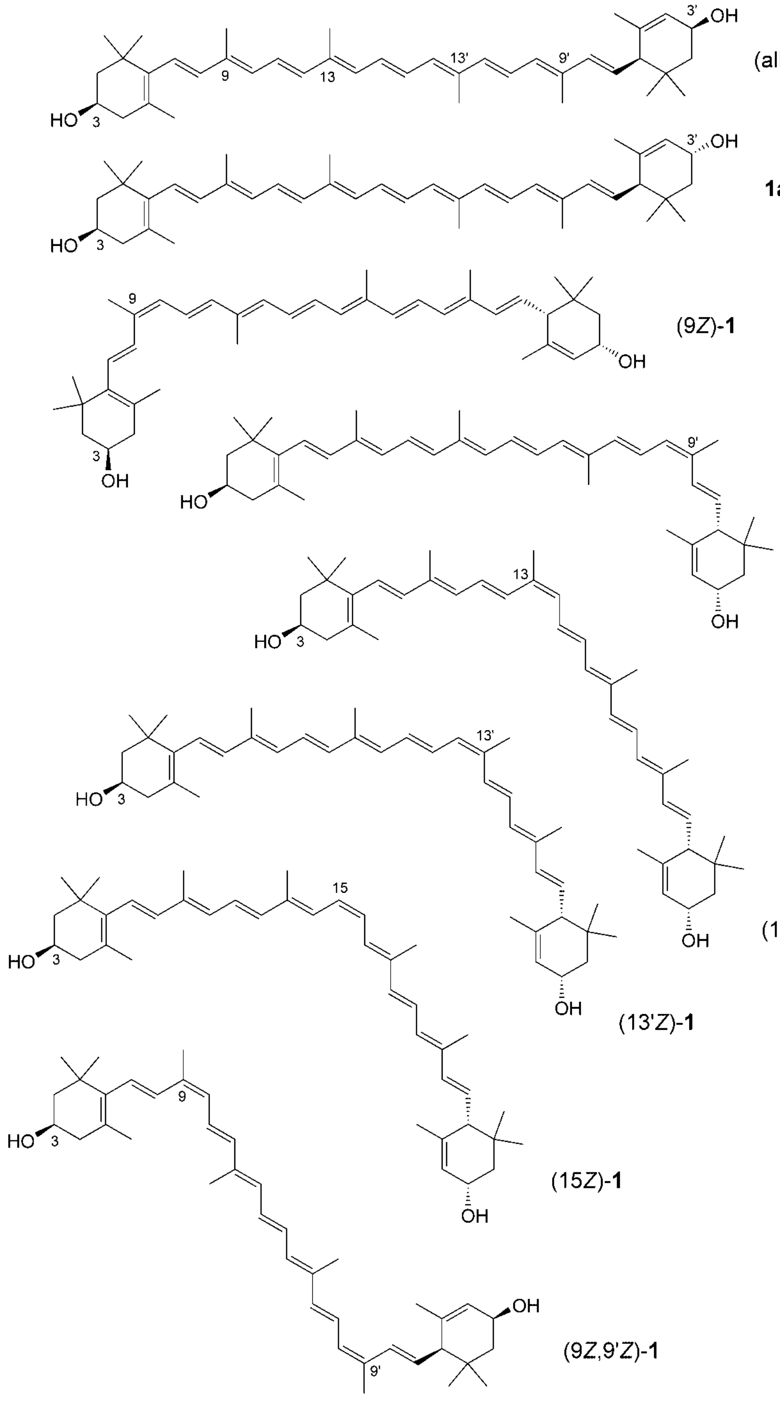
3′-Epilutein (=(all-*E*,3*R*,3′*S*,6′*R*)-4′,5′-didehydro-5′,6′-dihydro-*β*,*β*-carotene-3,3′-diol; **1**), isolated from the flowers of *Caltha palustris*, was submitted to both thermal isomerization and I_2-catalyzed photoisomerization. The structures of the main products (9*Z*)-**1**, (9′*Z*)-**1**, (13*Z*)-**1**, (13′*Z*)-**1**, (15*Z*)-**1,** and (9*Z*,9′*Z*)-**1** were determined based on UV/VIS, CD, ^{1}H-NMR, and MS data.

1. Introduction. – 3′-Epilutein (=(all-*E*,3*R*,3′*S*,6′*R*)-4′,5′-didehydro-5′,6′-dihydro-*β*,*β*-carotene-3,3′-diol; (all-*E*)-**1**), (13*Z*)-3′-epilutein ((13*Z*)-**1**), and (13′*Z*)-3′-epilutein ((13′*Z*)-**1**) have been isolated from the skin of trout [1]. Compounds (13*Z*)-**1** and (13′*Z*)-**1** have been prepared by (*E*/*Z*)-isomerization of (all-*E*)-**1**, and are also formed as by-products during the $NaBH_4$ reduction of 3′-oxolutein [1]. The chromatographic separation and spectroscopic characterization (^{1}H-NMR, CD, MS) of (13*Z*)-**1** and (13′*Z*)-**1** have also been published [1]. As the UV/VIS spectroscopic properties of (13*Z*)-**1**, (13′*Z*)-**1**, and (15*Z*)-**1**, as well as those of other stereoisomers of (all-*E*)-**1**, formed by thermal isomerization or I_2-catalyzed photoisomerization, had not been thoroughly examined, and since the preparation and structure elucidation of (9*Z*)-**1** and (9′*Z*)-**1** have not been published yet, we aimed at their UV/VIS spectroscopic characterization. We were also interested in determining the equilibrium composition of stereoisomers obtained from (all-*E*)-**1**, and wanted to prepare and characterize (9*Z*)-**1**, (9′*Z*)-**1**, and (9*Z*,9′*Z*)-**1** and confirm the absolute configuration of (13*Z*)-**1** and (13′*Z*)-**1** for comparison with the corresponding literature data [1].

In accordance with the investigations of *Eugster* and co-workers [2], we isolated from the flowers of *Caltha palustris* (all-*E*)-**1** on a preparative scale in the form of highly pure crystals [3]. The thermal isomerization and I_2-catalyzed photoisomerization [4] of lutein (**1a**) have already been investigated earlier during our systematic research concerning the (*E*/*Z*)-isomerization of the polyene chain of carotenoids [5]. The crystalline (9*Z*)-, (9′*Z*)-, (13*Z*)-, and (13′*Z*)-isomers of **1a** were prepared by (*E*/*Z*)-isomerization, and the geometrical configurations of the isomers were determined by means of spectroscopic methods [5].

As part of our systematic investigation of carotenoids [6–18], we now report the preparation and structure elucidation of the isomerization products of (all-*E*)-**1**. The resulting four main (mono-*Z*)-isomers, *i.e.*, (9*Z*)-**1**, (9′*Z*)-**1**, (13*Z*)-**1**, (13′*Z*)-**1**, as well as (9*Z*,9′*Z*)-3′-epilutein ((9*Z*,9′*Z*)-**1**), were prepared in purities higher than 95%



(all-*E*)-**1**

1a

(9*Z*)-**1**

(9'*Z*)-**1**

(13*Z*)-**1**

(13'*Z*)-**1**

(15*Z*)-**1**

(9*Z*,9'*Z*)-**1**

according to HPLC analysis, and were characterized by UV/VIS, circular dichroism (CD), ^{1}H-NMR, and mass spectroscopy (MS). Thereby, the (*Z*)-isomers of 3′-epilutein were prepared in view of their use as reference compounds for the identification of new, naturally occurring (*Z*)-carotenoids [19]. Moreover, the comparison of the (*E*/*Z*)-isomerization of carotenoids with different end groups may help identifying the structure of naturally occurring (*Z*)-isomers.

2. Results. – 2.1. *Thermal Isomerization.* The thermal isomerization of **1** (benzene, 80°, 2 h) gave, in agreement with the pioneering studies of *Zechmeister* [4] and others [5–18], (13*Z*)-**1** and (13′*Z*)-**1** as the main products. In addition (15*Z*)-**1**, (9*Z*)-**1**, and (9′*Z*)-**1**, or a mixture of the latter two isomers, were observed in small quantities. The composition of the quasi-equilibrium mixture was determined by preparative column chromatography (see *Table 1* and *Exper. Part*). The fractions of the corresponding isomers were crystallized from benzene/hexane 1:5, and the purity of all compounds was determined by HPLC.

2.2. *Iodine-Catalyzed Photoisomerization.* In agreement with previous results for other carotenoids [5–18], and, in accordance with the configurational asymmetry of 3′-epilutein ((all-*E*)-**1**), its I_2-catalyzed photoisomerization afforded (9*Z*)-**1**, (9′*Z*)-**1**, (13*Z*)-**1**, and (13′*Z*)-**1** as the main products. In addition, this thermodynamic-equilibrium mixture [14] contained trace amounts of (15*Z*)-**1**, (9*Z*,9′*Z*)-**1**, and two fractions (*Fr. A* and *Fr. B*) of other (di-*Z*)-isomers (see *Table 1* and *Sect. 4.2* in the *Exper. Part*). Again, the (*Z*)-isomers were separated chromatographically on a preparative scale and were then crystallized from benzene/hexane 1:5.

2.3. *Spectroscopic Characterization.* A tentative determination of the (*Z*)-configured C=C bond within the polyene chains was performed first by UV/VIS spectroscopy. The UV/VIS spectra of all geometrical isomers of 3′-epilutein, *i.e.*, (9*Z*)-**1**, (9′*Z*)-**1**, (13*Z*)-**1**, (13′*Z*)-**1**, (15*Z*)-**1**, (9*Z*,9′*Z*)-**1**, and two (di-*Z*)-isomers showed main absorption maxima between λ_{max} 488 – 475 nm (in benzene), in agreement with a nonene chromophore. The shape of the absorption bands indicated the presence of β- and ε-type end groups for all isomers. The characteristic UV/VIS data are presented in *Table 1.*

The presence of a weak '*cis*-peak' and a small hypsochromic shift of $\Delta\lambda_{max}$ of 5–6 nm relative to (all-*E*)-**1** is characteristic of (9*Z*)-**1** and (9′*Z*)-**1** (see *Table 1* and *Fig. 1*), in agreement with geometrical isomers containing a (*Z*)-configured C=C bond in a peripheral position [4][7][10][11]. The presence of a weak '*cis*-peak', in combination with a more-significant hypsochromic shift of $\Delta\lambda_{max}$ 10–12 nm, is characteristic of (9*Z*,9′*Z*)-**1**, which contains two (*Z*)-configured C=C bonds in peripheral positions [4][7][10][11]. Compounds (13*Z*)-**1** and (13′*Z*)-**1** exhibited a strong '*cis*-peak' at *ca.* 339–340 nm, with considerable $\Delta\lambda_{max}$ values of 7–9 nm, characteristic of isomers with a (*Z*)-configured C=C bond in a more central position of the polyene chain (*Table 1*, *Fig. 2*) [4][7][10][11]. Compound (15*Z*)-**1** exhibited a very strong '*cis*-peak' and small hypsochromic shift values ($\Delta\lambda_{max}$ = 3–4 nm). These data are characteristic of (*Z*)-isomers with a C=C bond in central position of the polyene chain (*Table 1*, *Fig. 2*) [4][6][7][10][11].

The configurations of the C=C bonds of the polyene chain in (all-*E*)-**1**, (9*Z*)-**1**, (9′*Z*)-**1**, (13*Z*)-**1**, (13′*Z*)-**1**, and (9*Z*,9′*Z*)-**1** were determined by ^{1}H-NMR spectroscopy,

Table 1. *Composition and UV/VIS Spectral Data of the Equilibrium Mixtures Obtained by Thermal Isomerization and by I_2-Catalyzed Photoisomerization of (all-*E*)-***1**

Compound	Distribution [%]	λ_{max} [nm] [a]	$A_{(Z)}/A_{max}$ [%] [b]
Thermal isomerization:			
(all-*E*)-**1**	72	488, 458, 434, 336	6
(13*Z*)-**1**	12	480, 451, 428, 338	42
(13′*Z*)-**1**	11	479, 451, 426, 340	39
(15*Z*)-**1**	3	484, 455, 431, 339	53
(9*Z*,9′*Z*)-**1**	2	481, 452, 430, 337	13
Photoisomerization:			
(all-*E*)-**1**	47	488, 458, 434, 336	6
(9*Z*)-**1**	14	481, 452, 428, 337	13
(9′*Z*)-**1**	11	483, 453, 431, 337	14
(13*Z*)-**1**	7	479, 451, 428, 338	42
(13′*Z*)-**1**	9	479, 451, 426, 340	39
(15*Z*)-**1**	1	484, 455, 431, 339	53
(9*Z*,9′*Z*)-**1**	1	476, 448, 426, 336	10
Fr. A [c]	5	476, 448, 425, 437	35
Fr. B [c]	7	475, 450, 420, 340	43

[a]) In C_6H_6 at ambient temperature. [b]) Absorbance ratio. [c]) Mixtures of sterically unhindered (di-*Z*)-isomers other than (9*Z*,9′*Z*)-**1** (see *Sect. 4.2* in the *Exper. Part*).

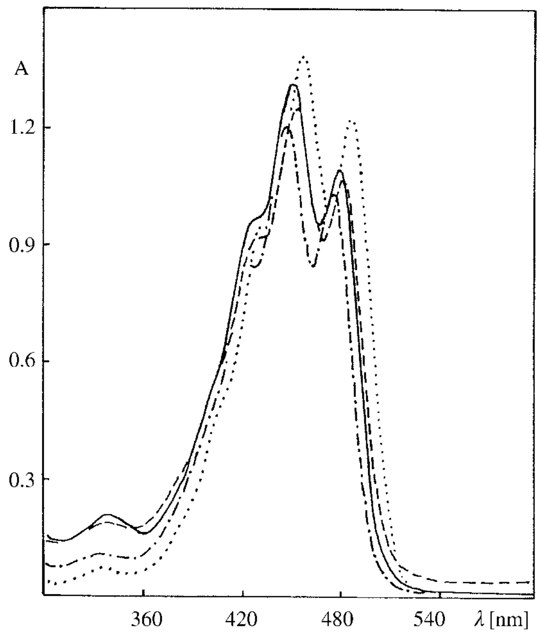


Fig. 1. *UV/VIS Spectra of (9*Z*)-***1** (—), *(9′*Z*)-***1** (– – –), *(9*Z*,9′*Z*)-***1** (– · – · – · –), *and (all-*E*)-***1** (·······). Recorded in C_6H_6 at room temperature.

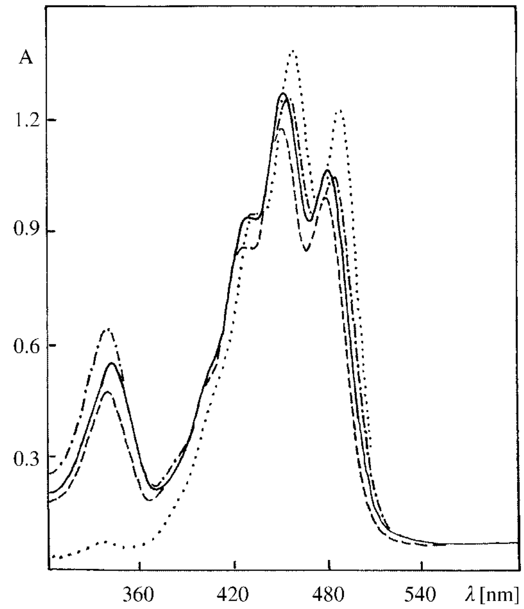


Fig. 2. *UV/VIS Spectra of (13Z)-**1** (—), (13′Z)-**1** (– – –), (15Z)-**1** (– · – · – · –), and (all-E)-**1*** (······). Recorded in C_6H_6 at room temperature.

and corroborated by ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-gHSQC measurements [20–22]. The data given in the *Exper. Part* and the characteristic ^{1}H-NMR isomerization shifts (*Table 2*) were identical to the corresponding data in the literature [1][21][22] and, thus, confirmed the structures of the investigated (*Z*)-isomers of 3′-epilutein.

Both lutein and 3′-epilutein are prominent representative 'heterodichiral' carotenoids. As classified in the literature, the CD spectra of these compounds are close to conservative [23–26]. It is known that conservative CD spectra are sensitive to (*E*/*Z*)-isomerization, and the CD bands of the (all-*E*)- and the (mono-*Z*)-isomers are typically opposite in sign [23–26].

The CD spectra of (9*Z*)-**1**[1]), (13*Z*)-**1**, and (13′*Z*)-**1** presented in *Fig. 3* were in full agreement with the above statement: between 260 and 370 nm, the *Cotton* effects of these (*Z*)-isomers were roughly mirror images of the corresponding bands of (all-*E*)-**1**. This sign inversion is due to the inversion of the direction of the twist imparted on the conjugated chain by the chiral end-groups [27]. In this respect, the whole carotenoid chromophore, including both the polyene chain and the conjugated s-*cis* C=C bond of one cyclohexene ring, becomes intrinsically chiral due to the twist caused by the asymmetric rings. According to the C_2-chirality rule, signs of *Cotton* effects observed between 250 and 370 nm suggest that the overall helicity of (all-*E*)-**1** is left-handed [26]. In the mono-(*Z*)-isomers, however, the twist of the end-groups with respect to each

1) Unfortunately, the CD spectrum of (9′*Z*)-**1** could not be recorded due to partial decomposition of the sample (trace amount) either during storage or measurement.

Table 2. *[1]H-NMR Isomerization Shifts of Mono- and Di-(Z)-Configured Isomers of 3′-Epilutein*. The $\Delta\delta(Z)$ values refer to the differential shift of selected signals of the (Z)-isomer relative to those of (all-E)-**1** ($\delta(Z) - \delta$(all-E)). All spectra (400 MHz) were recorded at room temperature in $CDCl_3$. The most-signifcant $\Delta\delta$ values are highlighted bold.

Group	$\Delta\delta(9Z)$)	$\Delta\delta(9'Z)$)	$\Delta\delta(13Z)$)	$\Delta\delta(13'Z)$	$\Delta\delta(9Z,9'Z)$
H–C(7)	0.01	0.01	0.03	0.00	0.00
H–C(8)	**0.53**	0.01	0.00	0.01	**0.53**
H–C(10)	– 0.08	0.01	0.06	– 0.02	– 0.07
H–C(11)	0.09	0.00	0.00	0.00	0.08
H–C(12)	– 0.06	0.00	**0.53**	0.01	– 0.06
H–C(14)	0.00	0.00	– 0.14	– 0.02	0.00
H–C(15)	– 0.01	0.00	0.17	– 0.07	0.00
Me(16)	0.02	0.01	0.02	0.01	0.02
Me(17)	0.03	0.01	0.02	0.01	0.03
Me(18)	0.04	0.00	0.01	0.00	0.04
Me(19)	0.00	0.01	0.03	0.00	0.01
Me(20)	0.00	0.01	0.02	0.01	0.01
H–C(7′)	0.01	0.02	0.00	0.03	0.02
H–C(8′)	0.00	**0.54**	0.02	0.01	**0.54**
H–C(10′)	0.00	– 0.11	0.02	0.06	– 0.11
H–C(11′)	0.03	0.17	0.03	0.04	0.18
H–C(12′)	0.00	– 0.06	0.00	**0.53**	– 0.06
H–C(14′)	0.00	0.00	0.00	– 0.13	0.00
H–C(15′)	0.00	0.00	– 0.06	0.16	0.00
Me(16′)	0.01	0.02	0.01	0.01	0.01
Me(17′)	0.01	0.03	0.01	0.02	0.03
Me(18′)	0.01	0.04	0.01	0.01	0.04
Me(19′)	0.00	0.00	0.00	0.01	0.00
Me(20′)	0.00	0.03	0.00	0.03	0.03

other becomes right-handed, thus reversing the helicity of the chromophore, which rationalizes the inverted signs of the CD bands. In contrast, the (9Z,9′Z)-isomer showed a very similar CD spectrum to that of (all-E)-**1**. This result can also be understood by the chiral influence of the terminal rings. Isomerization affects both ends of the molecule and occurs at equivalent bonds, *i.e.*, in 9- and 9′-position, leaving the overall symmetry of the molecule intact [27].

It is worth mentioning that CD spectra of (mono-Z)- and (di-Z)-isomers of 3′-epilutein provide additional evidence for the significant stereoelectronic interaction between the allylic C=C bond of the ε-ring and the polyene chain [23–25]. Due to the lack of conjugation between the polyene chain and the ε-ring, rotation about the C(6′)–C(7′) single bond seems to be unrestricted. Accordingly, for carotenoids carrying ε-ring(s), the CD spectra are expected to be weak and of the nonconservative type. Furthermore, if the effect of the ε-ring on the helicity of the chromophore is negligible in 3′-epilutein, then the molecule should behave as if it possessed only one end-ring, *i.e.*, here, a β-ring. Such molecules, however, should not show any sign inversion in their CD spectra upon (E/Z)-isomerization [27]. However, the contrary was observed, which indicates *1*) that there is a significant interaction between the polyene chain and the conjugated-ring-C=C bond, and *2*) that the helicity imposed by the β- and ε-rings on the chromophoric system follows the same sense.

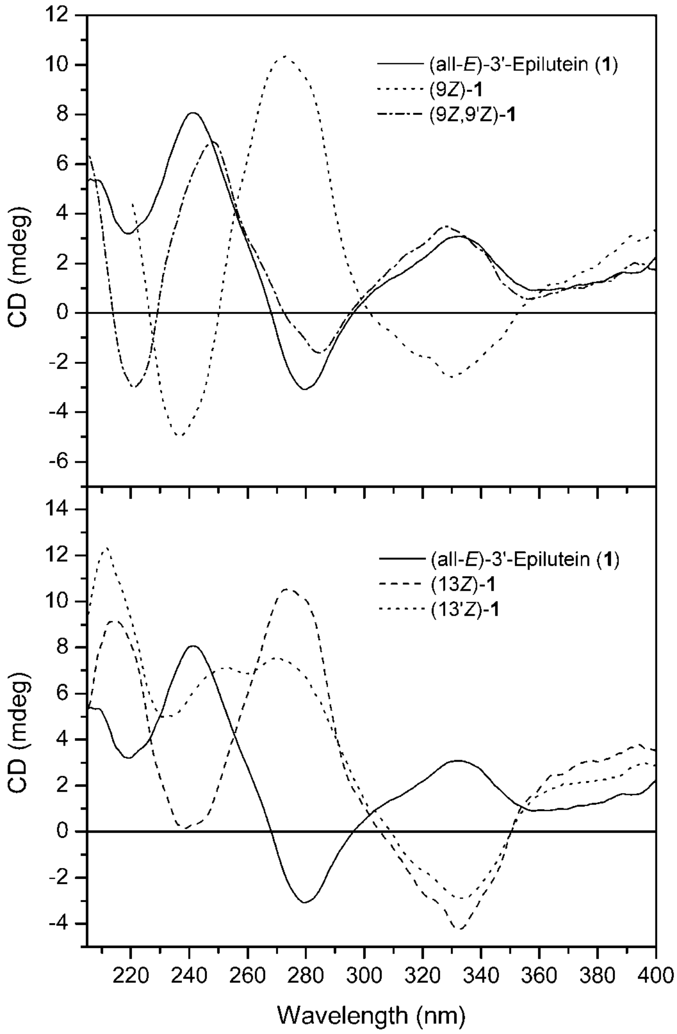


Fig. 3. *CD Spectra of the investigated geometrical isomers of 3′-epilutein* (**1**). Recorded in EtOH at room temperature.

Additionally, the CD spectra of the main (mono-*Z*)-isomers (9*Z*)-, (13*Z*)-, and (13′*Z*))-**1** of 3′-epilutein were found to be almost identical to the corresponding main (mono-*Z*)-isomers of lutein (**1a**) [23]. This suggested that the absolute configuration at C(3′) does not influence the spatial disposition between the C=C bond of the ε-ring and the polyene chain [23–26].

The mass spectra of all (*Z*)-isomers reported showed the corresponding molecular-ion peak at *m*/*z* 568 (M^+; $C_{40}H_{56}O_2^+$), together with signals at *m*/*z* 550 ($[M - H_2O]^+$) and 476 ($[M - C_7H_8]^+$) [2][28]. The detailed EI-MS data of the investigated (*Z*)-isomers are given in the *Exper. Part*.

3. Discussion. – The thermal (*E*/*Z*)-isomerization of 3′-epilutein ((all-*E*)-**1**) gave mainly the corresponding (13*Z*)- and (13′*Z*)-isomers and turned out to be a suitable method for the preparation of these compounds. The I_2-catalyzed photoisomerization of (all-*E*)-**1** resulted in complex mixtures of (*Z*)-isomers that were difficult to separate. As main products, the (mono-*Z*)-isomers (9*Z*)-**1**, (9′*Z*)-**1**, (13*Z*)-**1**, and (13′*Z*)-**1** were identified, together with one out of the six theoretically possible, sterically not hindered (di-*Z*)-isomers [4], *i.e.*, (9*Z*,9′*Z*)-**1**.

The structures of the main isomerization products of (all-*E*)-**1** correspond to the isomerization products of other carotenoids with a 3-OH group at the ε-ring of this chromophore [5][7–11][29].

The isomer composition of the equilibrium mixtures obtained from **1a** [5][7][10][11] and from **1** [30] were found to be nearly identical, showing that configurational differences have no significant influence on the process of stereomutation [18]. In addition, each individual (*Z*)-isomer was converted back to the parent compound, (all-*E*)-**1**, by I_2-promoted photoisomerization [4][7][9–11]. Also, the composition of the stereoisomeric equilibrium mixtures were very similar to those of carotenoids with the 3-OH group at the ε-ring, irrespective of the structure of the second end group (3-OH-β- or 3-OH-5,6-epoxy-5,6-dihydro-β-end group) [5][8].

The (*E*/*Z*)-isomerization rate for (all-*E*)-**1** was in the same order as for **1a** and other carotenoids containing a 3-OH group at the ε-ring [5][7–11][29]. The equilibrium was reached by thermal isomerization or by I_2-catalyzed photoisomerization within 120 min and 40 min, respectively [6][7][9–12].

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Experimental Part

1. *General.* See [3]. For circular dichroism, λ_{max} ('pos. max.'), λ_{min} ('neg. max.'), and λ_0 ('zero crossing') are given in nm.

2. *Thermal Isomerization.* A soln. of 3′-epilutein (**1**; 60 mg, isolated from the flowers of *Caltha palustris* [3][31]), in benzene (600 ml) was refluxed under N_2 in the dark, and isomerization was monitored by UV/VIS spectroscopy [4][7][11][29]. When the quasi-equilibrium was reached after *ca.* 120 min, the mixture was submitted to column-chromatographic purification (CC).

3. *I_2-Catalyzed Photoisomerization.* A soln. of 3′-epilutein (**1**; 40 mg) in benzene (400 ml) was isomerized at r.t. under N_2 in scattered daylight in the presence of I_2 (1 mg, *ca.* 0.02 equiv.) [4][7][9][10]. The isomerization was monitored by UV/VIS spectroscopy [4][7][10]. When the thermodynamic equilibrium was reached after *ca.* 40 min, the mixture was washed with 5% $Na_2S_2O_3$ soln. to remove I_2, and, after the usual workup [32], was submitted to CC.

4. *Separation of (*E/Z*)-Isomers.* The equilibrium mixtures were separated by CC (6 cm × 30 cm column, $CaCO_3$ (*Biogal*, Hungary)) and thoroughly characterized (see *Sect.* 5).

4.1. *Thermal-Isomerization Mixture.* Fourfold CC (benzene/hexane 2 : 1) of **1** resulted, after development, in the following picture (from top to bottom): 4-mm pale brownish-yellow zone (unidentified degradation products); 4-mm zone of intermediate; 25-mm yellow (= *Zone 1*; (all-*E*)-**1**); 2-mm zone of intermediate; 4-mm pale-yellow zone (= *Zone* 2; (9*Z*)-**1**); 10-mm zone of intermediate; 10-mm yellow zone (= *Zone* 3; (13*Z*)-**1**); 3-mm zone of intermediate; 4-mm pale-yellow zone (= *Zone* 4; (15*Z*)-**1**); 15-mm zone of intermediate; 15-mm yellow zone (= *Zone* 5; (13′*Z*)-**1**).

4.2. *I_2-Catalyzed Photoisomerization Mixture*. Threefold CC (benzene/hexane 1:1) of **1** resulted, after development, in the following picture (from top to bottom): 3- to 4-mm brownish-yellow zone (unidentified degradation products); 3-mm zone of intermediate; 5-mm pale-yellow zone (= *Zone 1*, *Fr. A*; (di-*Z*)-**1**); 5-mm zone of intermediate, 30-mm yellow zone (= *Zone* 2; (all-*E*)-**1**); 3-mm zone of intermediate; 8-mm pale-yellow zone (= *Zone* 3; (9*Z*,9′*Z*)-**1**); 4-mm zone of intermediate; 10-mm yellow zone (= *Zone* 4; (9*Z*)-**1**); 3-mm zone of intermediate; 6-mm yellow zone (= *Zone* 5; (13*Z*)-**4**); 2-mm zone of intermediate, 3-mm pale-yellow zone (= *Zone* 6; (15*Z*)-**1**); 6-mm zone of intermediate; 5-mm pale-yellow zone (= *Zone* 7, *Fr. B*; (di-*Z*)-**1**); 5-mm zone of intermediate, 8-mm yellow zone (= *Zone* 8; (13′*Z*)-**1**); 3-mm zone of intermediate; 12-mm yellow zone (= *Zone* 9; (9′*Z*)-**1**). After the usual workup [32], the corresponding fractions were combined, and the isomers were crystallized from benzene/hexane 1:5 to afford (all-*E*)-**1** (15 mg), (9*Z*)-**1** (4 mg), (9′*Z*)-**1** (3.8 mg), (9*Z*,9′*Z*)-**1** (0.8 mg), (13*Z*)-**1** (6 mg), (13′*Z*)-**1** (5.2 mg), and (15*Z*)-**1** (0.2 mg).

5. *Compound Characterization*. 5.1. *(all-*E*,3*R*,3′*S*,6′*R*)-4′,5′-Didehydro-5′,6′-dihydro-β,β-carotene-3,3′-diol* ((all-*E*)-**1**). Purity (HPLC): 98%. M.p. 148–150°. UV/VIS (benzene): see *Table 1*, *Figs. 1* and *2*. CD (EtOH; see *Fig. 3*): 217.5 (pos. min.), 241.5 (pos. max.), 268 (zero crossing), 280.5 (neg. min.), 296 (zero crossing), 332 (pos. max.). ^{1}H- and ^{13}C-NMR: see [3]. EI-MS: see [3].

5.2. *(3*R*,3′*S*,6′*R*,9*Z*)-4′,5′-Didehydro-5′,6′-dihydro-β,β-carotene-3,3′-diol* ((9*Z*)-**1**). Purity (HPLC): 95%. M.p. 87–89°. UV/VIS (benzene): see *Table 1* and *Fig. 1*. CD (EtOH; see *Fig. 3*): 226.5 (zero crossing), 237.5 (neg. min.), 250 (zero crossing), 272.5 (pos. max.), 302 (zero crossing), 328.5 (neg. min.), 352.5 (zero crossing), 442 (pos. max.), 459.5 (pos. min.), 469 (pos. max.). ^{1}H-NMR (400 MHz, $CDCl_3$)[2]): 0.85 (*s*, Me(16′)); 0.94 (*s*, Me(17′)); 1.08 (*s*, Me(16)); 1.09 (*s*, Me(17)); 1,39 (^2J = 12.9, *J*(2′ax,3′) = n.a., H_{ax}–C(2′)); 1.49 (*t*-like, ^2J = *J*(2eq,3) = 12.0, H_{eq}–C(2)); 1.64 (*dd*, *J*(2′eq,3′) = 10.2, H_{eq}–C(2′)); 1.64 (*s*, Me(18′)); 1.77 (*s*, Me(18)); 1.79 (*J*(2ax,3) = n.a., H_{ax}–C(2)); 1.90 (*s*, Me(19′)); 1.95 (*s*, Me(20)); 1.95 (*s*, Me(20′)); 1.96 (*s*, Me(19)); 2.07 (*dd*, ^2J = 16.9, *J*(4eq,3) = 9.0, H_{eq}–C(4)); 2.16 (*d*, *J*(6′,7′) = 9.4, H–C(6′)); 2.41 (^2J = 16.9, *J*(4ax,3) = 5.4, H_{ax}–C(4)); 4.02 (*m*, H–C(3)); 4.22 (*m*, H–C(3′)); 5.48 (br. *s*, H–C(4′)); 5.53 (*dd*, *J*(6′,7′) = 9.4, *J*(7′,8′) = 15.3, H–C(7′)); 6.06 (*d*, *J*(10,11) = 11.7, H–C(10)); 6.10 (*J*(7,8) = n.a., H–C(7)); 6.12 (*m*, *J*(8′,7′) = n.a., H–C(8′)); 6.12 (*J*(10′,11′) = n.a., H–C(10′)); 6.23 (*m*, H–C(14′)); 6.25 (*m*, H–C(14)); 6.29 (*d*, *J*(11,12) = 15.1, H–C(12)); 6.34 (*d*, *J*(11′,12′) = 15.1, H–C(12′)); 6.60 (*J*(11′,10′) = n.a., H–C(11′)); 6.61 (*m*, H–C(15)); 6.62 (*m*, H–C(15′)); 6.65 (*d*, *J*(8,7) = 15.7, H–C(8)); 6.73 (*dd*, *J*(10,11)) = 11.7, *J*(11,12) = 15.1, H–C(11)). For characteristic ^{1}H-NMR isomerization shifts, see *Table 2*. EI-MS: 568 (100, M^+), 550 (13, $[M-H_2O]^+$), 537 (2), 476 (4, $[M-C_7H_8]^+$), 392 (9), 223 (9), 209 (4), 183 (20), 157 (12), 156 (28), 145 (8), 133 (10), 119 (8), 95 (6), 69 (4), 57 (7), 43 (9).

5.3. *(3*R*,3′*S*,6′*R*,9′*Z*)-4′,5′-Didehydro-5′,6′-dihydro-β,β-carotene-3,3′-diol* ((9′*Z*)-**1**). Purity (HPLC): 94%. M.p. 87–88°. UV/VIS (benzene): see *Table 1* and *Fig. 1*. ^{1}H-NMR (400 MHz, $CDCl_3$)[2]): 0.86 (*s*, Me(16′)); 0.96 (*s*, Me(17′)); 1.07 (*s*, Me(16)); 1.07 (*s*, Me(17)); 1.41 (*dd*, ^2J = 12.8, *J*(2′ax,3′) = 9.8, H_{ax}–C(2′)); 1.47 (*t*-like, ^2J = 12.0, *J*(2eq,3) = 12.0, H_{eq}–C(2)); 1.66 (^2J = n.a., *J*(2′eq,3′) = n.a., H_{eq}–C(2′)); 1.67 (*s*, Me(18′)); 1.73 (*s*, Me(18)); 1.76 (*ddd*, ^2J = 12.0, *J*(2ax,3) = 3.5, *J* = 2.0, H_{ax}–C(2)); 1.90 (*s*, Me(19′)); 1.96 (*s*, Me(20)); 1.97 (*s*, Me(19)); 1.98 (*s*, Me(20′)); 2.04 (*dd*, ^2J = 16.8, *J*(4eq,3) = 9.4, H_{eq}–C(4)); 2.21(*d*, *J*(6′,7′) = 9.7, H–C(6′)); 2.38 (^2J = 16.8, *J*(4ax,3) = 5.2, H_{ax}–C(4)); 3.99 (*m*, H–C(3)); 4.23 (*m*, H–C(3′)); 5.49 (br. *s*, H–C(4′)); 5.54 (*dd*, *J*(6′,7′) = 9.7, *J*(7′,8′) = 15.2, H–C(7′)); 6.01 (*d*, *J*(10′,11′) = 11.6, H–C(10′)); 6.10 (*AB*, H–C(7)); 6.13 (*AB*, H–C(8)); 6.15 (*d*, *J*(10,11) = 11.4, H–C(10)); 6.23 (*m*, H–C(14′)); 6.25 (*m*, H–C(14)); 6.28 (*d*, *J*(11′,12′) = 15.0, H–C(12′)); 6.35 (*d*, *J*(11,12) = 15.0, H–C(12)); 6.62 (*m*, H–C(15′)); 6.64 (*dd*, *J*(10,11) = 11.3, H–C(11)); 6.66 (*m*, H–C(8′)); 6.74 (*dd*, *J*(10′,11′) = 11.6, *J*(11′,12′) = 15.0, H–C(11′)). For ^{1}H-NMR isomerization shifts, see *Table 2*. EI-MS: 568 (100, M^+), 550 (13, $[M-H_2O]^+$), 537 (8), 523 (11), 509 (6), 476 (6, $[M-C_7H_8]^+$), 396 (5), 392 (4), 368 (30), 339 (7), 313 (20), 285 (80, 264 (12), 236 (21), 223 (7), 209 (8), 183 (7), 157 (8), 145 (12), 133 (7), 119 (9), 97 (9), 95 (8), 69 (8), 57 (11), 43 (12).

5.4. *(3*R*,3′*S*,6′*R*,9*Z*,9′*Z*)-4′,5′-Didehydro-5′,6′-dihydro-β,β-carotene-3,3′-diol* ((9*Z*,9′*Z*)-**1**). Purity (HPLC): 96%. M.p. 140–142°. UV/VIS (benzene): see *Table 1* and *Fig. 1*. CD (EtOH; see *Fig. 3*): 213.5 (zero crossing), 220.5 (neg. min.), 248 (pos. max.), 272.5 (zero crosing), 284.5 (neg. min.), 295 (zero crossing), 328.5 (pos. max.), 388 (pos. max.), 398 (pos. min.), 416 (pos. max.), 424.5 (pos. min.), 436.5 (pos. max.), 455.5 (pos. min.), 466.5 (pos. max.). ^{1}H-NMR (400 MHz, $CDCl_3$)[2]): 0.85 (*s*, Me(16′)); 0.96 (*s*, Me(17′)); 1.08 (*s*, Me (16)); 1.09 (*s*, Me(17)); 1.41 (^2J = n.a., *J*(2′ax,3′) = n.a., H_{ax}–C(2′)); 1.49 (*t*-like, ^2J = *J*(2eq,3) = 12.0, H_{eq}–C(2)); 1.66 (^2J = n.a., *J*(2′eq,3′) = n.a., H_{eq}–C(2′)); 1.67 (*s*, Me(18′)); 1.77 (*s*, Me(18)); 1.79 (*ddd*, ^2J = 12.0, *J*(2ax,3) = 3.4, *J* = 2.0, H_{ax}–C(2)); 1.90 (*s*, Me(19′)); 1.96 (*s*, Me(19)); 1.96 (*s*, Me(20)); 1.98 (*s*, Me(20′)); 2.08 (^2J = n.a., *J*(4eq,3) = n.a., H_{eq}–C(4)); 2.21 (*d*, *J*(6′,7′) = 9.6, H–C(6′)); 2.41 (*dd*, ^2J = 17.0, *J*(4ax,3) = 5.4, H_{ax}–C(4)); 4.02 (*m*, H–C(3));

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2) Abbreviation: n.a. means *not available*.

4.23 (*m*, H−C(3′)); 5.49 (br. *s*, H−C(4′)); 5.54 (*dd*, *J*(6′,7′) = 9.6, *J*(7′,8′) = 15.4, H−C(7′)); 6.01 (*d*, *J*(10′,11′) = 11.5, H−C(10′)); 6.07 (*d*, *J*(10,11) = 11.3, H−C(10)); 6.09 (*m*, H−C(7)); 6.12 (*m*, H−C(8)); 6.23 (*m*, H−C(14′)); 6.25 (*m*, H−C(14)); 6.27 (*d*, *J*(11′,12′) = 4.5, H−C(12′)); 6.30 (*d*, *J*(11,12) = 4.1, H−C(12)); 6.62 (*m*, H−C(15)); 6.62 (*m*, H−C(15′)); 6.66 (*m*, H−C(8′)); 6.72 (*dd*, *J*(10,11) = 11.3, *J*(11,12) = 4.1, H−C(11)); 6.75 (*dd*, *J*(10′,11′) = 11.5, *J*(11′,12′) = 4.5, H−C(11′)). For ^{1}H-NMR isomerization shifts, see *Table 2*. EI-MS: 568 (100, M^+), 550 (13, $[M - H_2O]^+$), 537 (5), 523 (6), 509 (3), 476 (5, $[M - C_7H_8]^+$), 392 (6), 368 (15), 339 (4), 313 (10), 285 (4), 264 (6), 236 (10), 223 (8), 209 (6), 183 (13), 157 (10), 145 (10), 133 (8), 119 (8), 95 (7), 69 (6), 57 (9), 43 (11).

5.5. *(3R,3′S,6′R,13Z)-4′,5′-Didehydro-5′,6′-dihydro-β,β-carotene-3,3′-diol* ((13*Z*)-**1**). Purity (HPLC): 92%. M.p. 86–87°. UV/VIS (benzene): see *Table 1* and *Fig. 2*. CD (EtOH; see *Fig. 3*): 214.5 (pos. max.), 238.5 (zero crossing), 273 (pos. max.), 305.5 (zero crossing), 332.5 (neg. min.), 350.5 (zero crosing), 436.5 (pos. max.). ^{1}H-NMR (400 MHz, $CDCl_3$)[2]): 0.85 (*s*, Me(16′)); 0.94 (*s*, Me(17′)); 1.08 (*s*, Me(16)); 1.08 (*s*, Me(17)), 1.39 (*dd*, ^2J = 12.0, *J*(2′ax,3′) = 9.9, H_{ax}−C(2′)); 1.48 (*t*-like, ^2J = 12.0, *J*(2eq,3) = 12.0, H_{eq}−C(2)); 1.63 (^2J = n.a., *J*(2′eq,3′) = n.a., H_{eq}−C(2′)); 1.64 (*s*, Me(18′)); 1.74 (*s*, Me(18)); 1.78 (*ddd*, ^2J = 11.8, *J*(2ax,3) = 3.5, *J* = 2.1, H_{ax}−C(2)); 1.90 (*s*, Me(19′)); 1.95 (*s*, Me(20′)); 1.97 (*s*, Me(20)); 1.99 (*s*, Me(19)); 2.05 (*dd*, ^2J = 17.1, *J*(4eq,3) = 9.7, H_{eq}−C(4)); 2.16 (*d*, *J*(6′,7′) = 9.3, H−C(6′)); 2.39 (*dd*, ^2J = 17.1, *J*(4ax,3) = 5.5, H_{ax}−C(4)); 4.00 (*m*, H−C(3)); 4.23 (*m*, H−C(3′)); 5.48 (br. *s*, H−C(4′)); 5.52 (*dd*, *J*(6′,7′) = 9.3, *J*(7′,8′) = 15.5, H−C(7′)); 6.11 (*m*, H−C(14)); 6.12 (*m*, H−C(7)); 6.12 (*m*, H−C(8)); 6.14 (*m*, H−C(8′)); 6.14 (*J*(10′,11′) = n.a., H−C(10′)); 6.20 (*d*, *J*(10,11) = 11.7, H−C(10)); 6.23 (*m*, H−C(14′)); 6.34 (*d*, *J*(11′,12′) = 15.0, H−C(12′)); 6.56 (*m*, H−C(15′)); 6.59 (*dd*, *J*(10′,11′) = 11.5, *J*(11′,12′) = 15.0, H−C(11′)); 6.64 (*dd*, *J*(10,11) = 11.7, *J*(11,12) = 14.8, H−C(11)); 6.79 (*m*, H−C(15)); 6.88 (*d*, *J*(11,12) = 14.8, H−C(12)). For ^{1}H-NMR isomerization shifts, see *Table 2*. EI-MS: 568 (100, M^+), 550 (9, $[M - H_2O]^+$), 476 (6, $[M - C_7H_8]^+$), 237 (2), 223 (6), 209 (7), 183 (4), 157 (7), 145 (11), 133 (7), 119 (8), 95 (6), 81 (3), 69 (3), 57 (5), 43 (6).

5.6. *(3R,3′S,6′R,13′Z)-4′,5′-Didehydro-5′,6′-dihydro-β,β-carotene-3,3′-diol* ((13′*Z*)-**1**). Purity (HPLC): 94%. M.p. 90–92°. UV/VIS (benzene): see *Table 1* and *Fig. 2*. CD (EtOH; see *Fig. 3*): 211 (pos. max), 230 (pos. min.), 253.5 (pos. max.), 260 (pos. min.), 270 (pos. max.), 309 (zero crossing), 333.5 (neg. min.), 350.5 (zero crossing), 412.5 (pos. max.), 425 (pos. min.), 437 (pos. max.), 457 (pos. min.), 467 (pos. max.). ^{1}H-NMR (400 MHz, $CDCl_3$)[2]): 0.85 (*s*, Me(16′)); 0.95 (*s*, Me(17′)); 1.07 (*s*, Me(16)); 1.07 (*s*, Me(17)); 1.39 (*dd*, ^2J = 12.4, *J* (2′ax,3′) = 9.9, H_{ax}−C(2′)); 1.48 (*t*-like, ^2J = 11.8, *J*(2eq,3) = 11.8, H_{eq}−C(2)); 1.64 (^2J = n.a., *J*(2′eq,3′) = n.a., H_{eq}−C(2′)); 1.64 (*s*, Me−C(18′)); 1.73 (*s*, Me−C(18)); 1.77 (*ddd*, ^2J = 11.8, *J*(2ax,3) = 3.7, *J* = 2.3, H_{ax}−C(2)); 1.91 (*s*, Me−C(19′)); 1.96 (*s*, Me−C(19)); 1.96 (*s*, Me−C(20)); 1.98 (*s*, Me−C(20′)); 2.04 (*dd*, ^2J = 17.1, *J*(4eq,3) = 9.5, H_{eq}−C(4)); 2.17 (*d*, *J*(6′,7′) = 9.5, H−C(6′)); 2.38 (*dd*, ^2J = 17.1, *J*(4ax,3) = 5.8, H_{ax}−C(4)); 4.00 (*m*, H−C(3)); 4.23 (*m*, H−C(3′)); 5.48 (br. *s*, H−C(4′)); 5.55 (*dd*, *J*(6′,7′) = 9.5, *J*(7′,8′) = 15.5, H−C(7′)); 6.09 (*AB*, H−C(7)); 6.10 (*m*, H−C(14′)); 6.12 (*J*(10,11) = n.a., H−C(10)); 6.13 (*AB*, H−C(8)); 6.13 (*m*, H−C(8′)); 6.18 (*J*(10′,11′) = n.a., H−C(10′)); 6.23 (*m*, H−C(14)); 6.36 (*d*, *J*(11,12) = 15.0, H−C(12)); 6.55 (*m*, H−C(15)); 6.60 (*dd*, *J*(10′,11′) = 11.5, *J*(11′,12′) = 15.1, H−C(11′)); 6.64 (*J*(10,11) = n.a., H−C(11)); 6.78 (*m*, H−C(15′)); 6.87 (*d*, *J*(11′,12′) = 15.1, H−C(12′)). For ^{1}H-NMR isomerization shifts, see *Table 2*. EI-MS: 568 (100, M^+), 550 (14, $[M - H_2O]^+$), 505 (1), 476 (2, $[M - C_7H_8]^+$), 430 (3), 299 (2), 275 (3), 237 (3), 223 (4), 209 (6), 183 (3), 159 (6), 145 (6), 119 (6), 95 (2), 89 (3), 69 (7), 57 (1), 55 (2), 45 (8), 43 (7).

5.7. *(3R,3′S,6′R,15Z)-4′,5′-Didehydro-5′,6′-dihydro-β,β-carotene-3,3′-diol* ((15*Z*)-**1**). Purity (HPLC; trace amount): 91%. M.p. 94–96°. UV/VIS (benzene): see *Table 1* and *Fig. 2* [4][7][10][11][33].

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