Preparation and Spectroscopic Characterization of (9*Z*,9′*Z*)-Lutein (Neolutein C)

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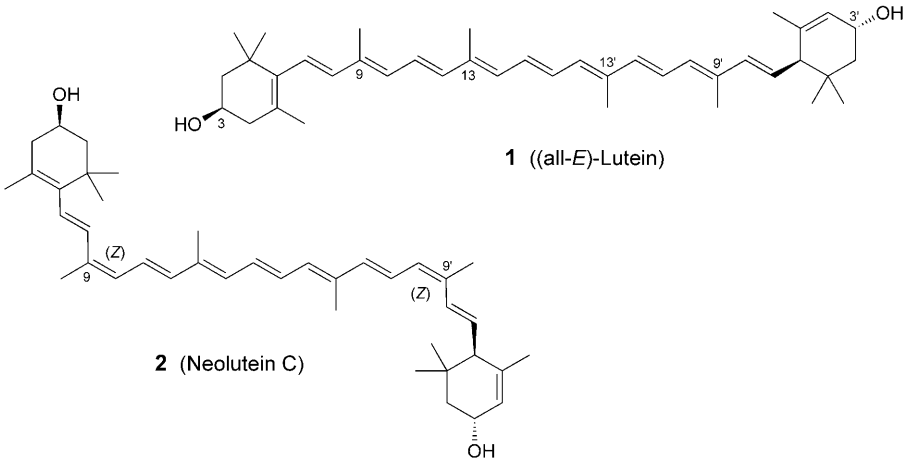
The preparation of pure, crystalline (9*Z*,9′*Z*)-lutein (neolutein C; **2**) by I_2-catalyzed photoisomerization of (all-*E*)-lutein (**1**) is described. The structure of **2** was unambiguously determined by UV/VIS, CD, and NMR spectroscopy, as well as by mass spectrometry, and the complete assignment of the ^{13}C-NMR spectrum of this carotenoid is presented for the first time.

Introduction. – Lutein (=(all-*E*,3*R*,3′*R*,6′*R*)-*β*,*ε*-carotene-3,3′-diol; **1**) is the main xanthophyll found in the major light-harvesting pigment–protein complex of higher plants, and involved in energy-transfer mechanisms during photosynthesis. In its fatty-acid-ester form, lutein (**1**) is widely distributed in fruits, blossoms or flowers, and yellow autumn leaves [1][2]. This carotenoid features a constitutionally asymmetric, (all-*E*)-configured decaene chromophore, with two structurally and sterically different end groups. According to the pioneering studies of *Zechmeister et al.* [3][4], the iodine-catalyzed photoisomerization of **1** yields two main isomeric products designated as neoluteins A and B (according to decreasing adsorption affinity towards $CaCO_3$). On the basis of UV/VIS spectroscopic data, these two neoluteins, respectively, were tentatively assigned by *Zechmeister* and co-workers as the (13*Z*)- or (15*Z*)-, and the (9*Z*)- and (9′*Z*)-isomers of lutein (**1**) [5]. In accordance with careful and systematic chromatographic studies carried out in our laboratory, it was established that the iodine-catalyzed stereomutation of constitutionally asymmetric carotenoids affords, by virtue of the non-equivalence of positions C(9) and C(9′), and C(13) and C(13′), *four* rather than two main mono-*cis* isomers [2][6].

In our earlier research on the (*E*/*Z*)-isomerization of C_{40} carotenoids, the main four (mono-*Z*)-isomers of lutein (**1**), *i.e.*, (9*Z*)-, (9′*Z*)-, (13*Z*)-, and (13′*Z*)-**1**, have been prepared in highly pure crystalline state, and their geometric configurations were determined by ^{13}C-NMR spectroscopy [2][7][8]. In continuation of our systematic investigations on the (*E*/*Z*)-isomerization of carotenoids [9–35], we herein report the preparation of crystalline (9*Z*,9′*Z*)-lutein (neolutein C; **2**) [5], and its characterization by UV/VIS, ^{1}H- and ^{13}C-NMR, and CD spectroscopy, as well as by mass spectrometry.

Results and Discussion. – Neolutein C (**2**) was prepared by iodine-catalyzed photoisomerization of (all-*E*)-lutein (**1**) [2–5][8][13][28]. Starting from 500 mg of **1**, the sep-



aration of the thermodynamic-equilibrium mixture [36][37] was performed by multiple preparative column chromatography on $CaCO_3$, eluting with benzene/hexane (see *Exper. Part*) [2][38]. After crystallization of the separated fractions, we obtained 10.0 mg of **2**. The HPLC profile of a mixture of **1** and **2** is shown in *Fig. 1*.

The UV/VIS spectrum of **2** in benzene (*Fig. 2*) showed λ_{max} values at 476, 446, 424, and 338 nm, with characteristic hypsochromic shifts ($\Delta\lambda_{max}$) of *ca.* 10–12 nm relative to **1**. The absorbance A of the *cis*-peak showed a $Q(A_{max}/A_{cis\text{-peak}})$ value of 9.21, and an

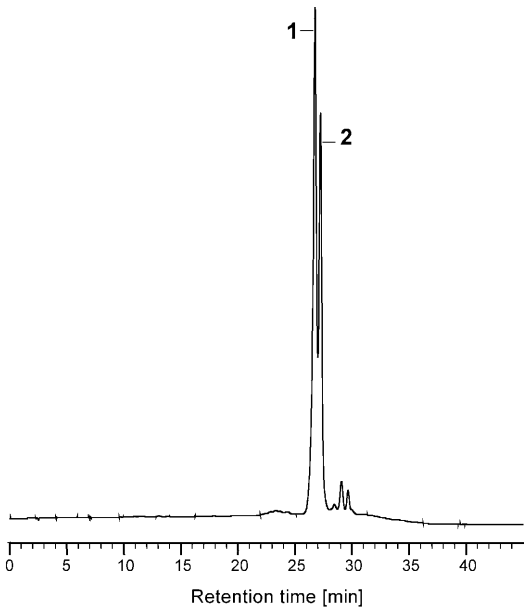


Fig. 1. *Reverse-Phase HPLC Separation of* (*all*-E)-*lutein* (**1**) *and* (9Z,9′Z)-*lutein* (**2**) *on a Chromsyl-C_{18} column with a H_2O/MeOH/acetone gradient*. For details, see *Exper. Part*.

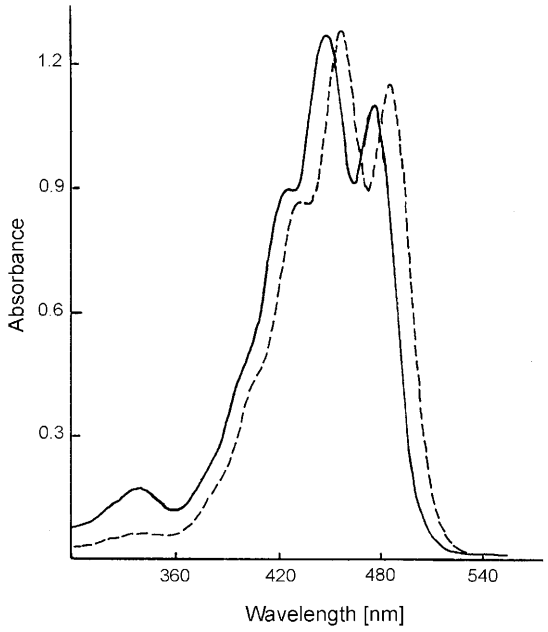


Fig. 2. *UV/VIS Spectra of* **1** (– – –) *and* **2** (——) *in benzene.* For the corresponding UV/VIS spectra in EtOH, see *Fig. 3,b.*

$A_{cis\text{-peak}}/A_{max}$ ratio of 10.86%. These data, together with the CD spectrum (see *Fig. 3,a* below), clearly indicated the (9*Z*,9′*Z*)-configuration for **2** [5][8][13][28].

The natural occurrence of (putative) neolutein C (**2**), and its isolation and structure elucidation from the petals of rape (*Brassica napus*), were published recently [39]. In comparison with (all-*E*)-lutein (**1**), the UV/VIS absorption maxima of the isolated (*Z*)-isomer were reported to be shifted by only 5 nm towards shorter wavelengths [39]. This value, however, is typical for (mono-*Z*)-isomers such as for the (9*Z*)- or (9′*Z*)-isomer, or their mixture [5][8][13][28]. We, thus, strongly suspect that the authors had, actually, isolated neolutein B, which is a *ca.* 1:1 mixture of (9*Z*)- and (9′*Z*)-lutein, rather than (9*Z*,9′*Z*)-lutein (**2**) proper. As mentioned above, the latter gives rise to much larger $\Delta\lambda_{max}$ values of *ca.* 10–12 nm (in various solvents). Except for this apparent difference, the ^{1}H-NMR, CD, and MS data of neolutein B and neolutein C (**2**) are identical.

The assignments of the ^{1}H- and ^{13}C-NMR signals[1]) of **2** were corroborated by ^{1}H,^{1}H-COSY, DEPT-135, gradient-enhanced ^{13}C,^{1}H-HSQC, ^{13}C,^{1}H-HMBC, and TROESY experiments, all performed with the standard *Varian* software. The characteristic ^{1}H- and ^{13}C-NMR data of **2** are presented in *Table 1*, and the ^{1}H- and ^{13}C-NMR 'isomerization shifts' ($\Delta\delta = \delta(Z) - \delta(\text{all-}E)$) are presented in *Table 2*. All NMR data were identi-

[1]) The ^{13}C-NMR data of **2** are reported here for the first time.

Table 1. *^{1}H- and ^{13}C-NMR Data of* **2**. At 400 or 100 MHz, resp., in $CDCl_3$ at ambient temperature.

Position	δ(H) [ppm]	*J* [Hz]	δ(C)	Position	δ(H) [ppm]	*J* [Hz]	δ(C)
1	–	–	37.07	3′	4.20–4.30 (*m*)	–	65.91
2	α: 1.78 (*ddd*)	n.a.[a])	48.35	4′	5.55 (br. *s*)	–	124.58
	β: 1.48 (*t*-like)	$^2J=^3J(2\beta,3)=12$					
3	3.90–4.10 (*m*)	–	65.08	5′	–	–	137.80
4	α: 2.40 (*dd*)	$^2J=16.9$	42.51	6′	2.46 (*d*)	$^3J(6',7')=10.1$	55.25
		$^3J(4\alpha,2)=5.3$					
	β: 2.06 (*dd*)	$^3J(4\beta,3)=9.6$					
5	–	–	126.35	7′	5.45 (*dd*)	$^3J(7',8')=15.4$	130.83
6	–	–	138.03	8′	6.65 (*d*)	n.a.	129.84
7	6.11 (*d*)	$^3J(7,8)=15.7$	127.38	9′	–	–	134.21[b])
8	6.65 (*d*)	n.a.	130.69	10′	6.01 (*d*)	$^3J(10',11')=11.5$	129.24
9	–	–	133.67[b])	11′	6.71–6.74 (*m*)	–	123.51[c])
10	6.06 (*d*)	$^3J(10,11)=11.6$	129.80	12′	6.28 (*d*)	$^3J(11',12')=15$	136.80[d])
11	6.71–6.74 (*m*)	–	123.65[c])	13′	–	–	136.29[e])
12	6.28 (*d*)	$^3J(11,12)=15.0$	136.85[d])	14′	6.21–6.23 (*m*)[f])	–	132.42[f])
13	–	–	136.23[e])	15′	6.59–6.62 (*m*)	–	129.92[g])
14	6.23–6.25 (*m*)[f])	–	132.44[f])	16′	1.01 (*s*)	–	29.53
15	6.59–6.62 (*m*)	–	129.97[g])	17′	0.85 (*s*)	–	24.83
16	1.07 (*s*)	–	30.29	18′	1.63 (*s*)	–	22.91
17	1.08 (*s*)	–	28.75	19′	1.90 (*s*)	–	12.11
18	1.76 (*s*)	–	21.73	20′	1.95 (*s*)	–	12.86[h])
19	1.96 (*s*)	–	20.74				
20	1.97 (*s*)	–	12.89[h])				
1′	–	–	33.97				
2′	α: 1.37 (*dd*)	$^2J=13.3$	44.76				
		$^3J(2'\alpha,3')=6.9$					
	β: 1.85 (*dd*)	$^3J(2'\beta,3')=5.9$					

[a]) Not available. [b])–[h]) Assignment may be exchanged.

cal with those reported in the literature [2][7][8][39][40], which confirmed the (9*Z*,9′*Z*)-configuration of the polyene chain.

Similar to (all-*E*)-lutein (**1**), the CD spectrum of (9*Z*,9′*Z*)-lutein (**2**) displays, below 275 nm, two positive maxima at 246 and 206.5 nm, and a minimum at 225.5 nm (*Fig. 3*,*a*). The 246-nm maximum exactly matches with the corresponding signal of **1**, but the high-energy CD band (207 nm) is at shorter wavelengths by 5 nm relative to the corresponding band for **1** (212 nm; positive *Cotton* effect). Additionally, the small negative CD band at 284 nm for **1** is absent in the spectrum of **2**.

Neolutein C (**2**) is a heterodichiral carotenoid with two distinct β- and ε-rings. In general, the CD spectroscopic features of homo- and, partly, of heterodichiral carotenoids are determined by the helical twist between the C=C bonds of the end groups when viewed along the linear polyene chain connecting them [25][41]. Upon formation of an *odd* number of (*Z*)-configured C=C bonds, the sign of this helical twist (clockwise or counterclockwise) inverts, which results in a CD spectrum of opposite sign compared to that of the (all-*E*)-isomer. When an *even* number of (*Z*)-configured C=C bonds is

Table 2. *^{1}H- and ^{13}C-NMR Isomerization Shifts of* **2** *Relative to* **1**

H-Atom	$\Delta\delta$(H)[a])	C-Atom	$\Delta\delta$(C)[a])
H–C(7)	0.00	C(7)	+2.46
H–C(8)	+0.52	C(8)	−7.79
H–C(10)	−0.10	C(9)	−2.00 or −1.47
H–C(11)	–	C(10)	−1.49
H–C(12)	−0.08	C(11)	−1.14 or −1.28
H–C(12′)	−0.08	C(12)	−0.69 or −0.74
H–C(11′)	–	C(12′)	−0.69 or −0.74
H–C(10′)	−0.13	C(11′)	−0.97 or −0.83
H–C(8′)	+0.52	C(10′)	−1.55
Me(19)	–	C(9′)	−0.84 or −1.38
Me(19′)	–	C(8′)	−7.87
		C(7′)	+2.12
		Me(19)	+8.00
		Me(19′)	+8.01

[a]) $\Delta\delta = \delta(Z) - \delta(\text{all-}E)$.

present, however, the sign of the helical twist is preserved, and the *cis*-isomer exhibits CD bands with the same signs as the corresponding (all-*E*) compound [41][42]. Obviously, the latter case is valid for (9*Z*,9′*Z*)-lutein (**2**), whose principal CD bands have the same sign as those of the parent all-*trans* form **1** (*Fig. 3*, *a*). Some minor differences observed between the two curves are due to the non-identical end groups.

The MS data of neolutein C (**2**) were in accordance with literature data [2][7][8][43]. The molecular-ion peak was observed at *m*/*z* 568 (M^+, $C_{40}H_{56}O_2^+$), with fragments at *m*/*z* 550 ($[M-H_2O]^+$), 476 ($[M-C_7H_8]^+$), 458, 237, 209, 197, 157, 145, 119, 105, 69, and 43.

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Experimental Part

General. Column chromatography (CC) was performed on $CaCO_3$ (*Biogal*, Hungary) with columns of 6×30 cm in size [38]. HPLC: *Dionex 580* pump, *HP 1050* detector with *HP* ChemStation software and *Waters 991* photodiode-array detector; *Chromsyl C$_{18}$* (6 μm; end-capped) column (250×4.6 mm i.d.); gradient elution (in linear steps, and at a flow rate of 1.25 ml/min) with solvents *A* (H_2O/MeOH 12:78), *B* (MeOH), and *C* (acetone/MeOH 1:1): 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*. UV/VIS Spectra: *Beckman DU-65* spectrophotometer; λ_{max} in nm. CD Spectra: *JASCO J-715* spectropolarimeter; λ in nm ($\Delta\varepsilon$ in M^{-1} cm^{-1}); in EtOH at r.t. NMR Spectra: *Varian Unity Inova 400-WB* spectrometer; at 400 (^{1}H) or 100 MHz (^{13}C); in $CDCl_3$ at 25°; chemical shifts δ in ppm rel. to Me_4Si (^{1}H) or to residual solvent signals (^{13}C). MS: *Varian MA-CH-7A* mass spectrometer; in *m*/*z* (rel. %).

Preparation of (9Z,9′Z)-Lutein (= *Neolutein C*; **2**). In accordance with literature procedures [2–5][8][13][28][35][36][37], a soln. of (all-*E*)-lutein (**1**; 500 mg) in benzene (2.5 l) was isomerized in the presence of I_2 (10 mg, 2%) in diffuse daylight. The thermodynamic-equilibrium mixture [36] was separated by repeated (20×) CC ($CaCO_3$; benzene/hexane 1:1) affording the following fractions (Fr.) in order of

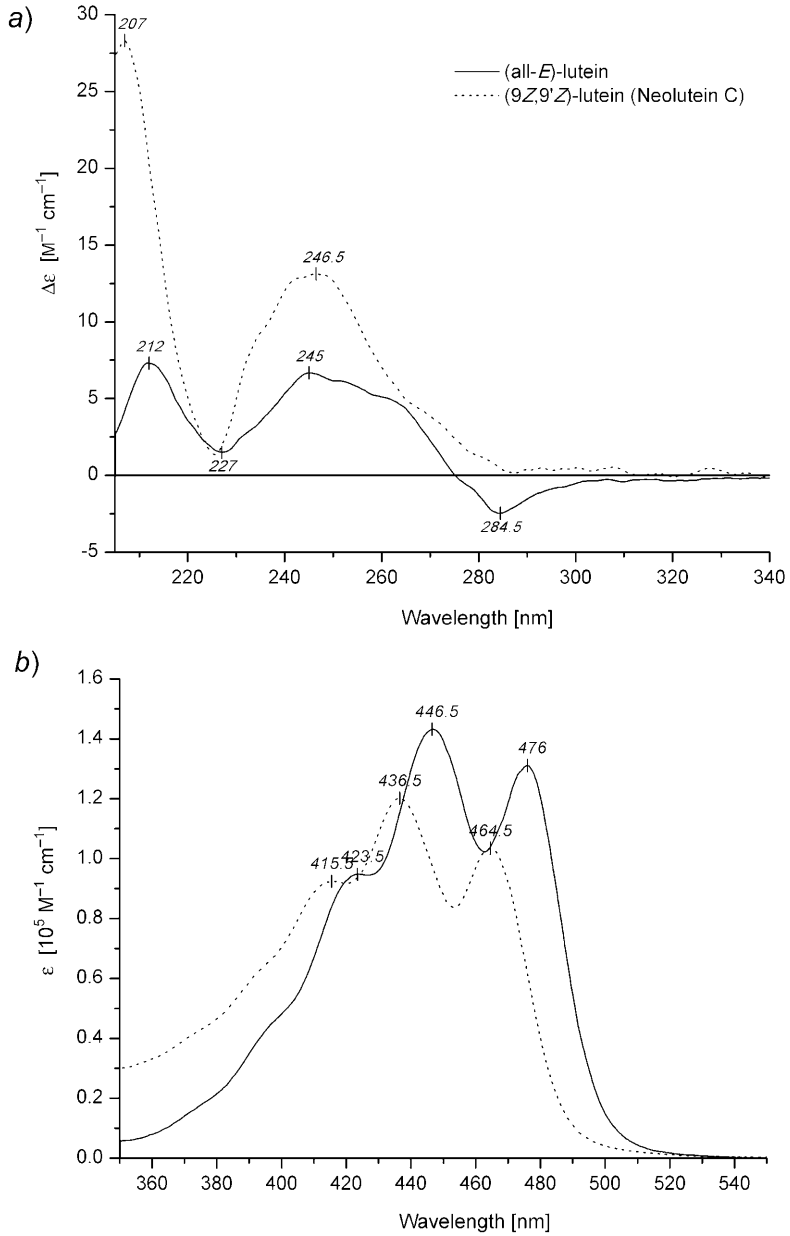

Fig. 3. *CD Spectra* (a) *and UV/VIS spectra* (b) *of* **1** *and* **2** *in EtOH*

decreasing adsorption affinity: *Fr. 1*: neolutein A = (13*Z*)-**1**/(13′*Z*)-**1**/(15*Z*)-**1**; *Fr. 2*: neolutein B = (9*Z*)-**1**/-(9′*Z*)-**1**; *Fr. 3*: neolutein C (**2**) = (9*Z*,9′*Z*)-**1**; *Fr. 4*: neoluteins D–F plus other (di-*Z*)-isomers of **1**; *Fr. 5*: (all-*E*)-lutein (**1**). The separated fractions were crystallized from benzene/hexane to afford 50 mg of neolutein A, 120 mg of neolutein B, 10 mg of **2**, 25 mg of neoluteins D–F *etc.*, and 200 mg of **1**.

Data of **2**. M.p. 182–184°. UV/VIS (benzene): 476, 446, 424, 338. UV/VIS (EtOH): 327.5, 415.5, 436.5, 464.5. CD (EtOH, r.t.): 207 (+28.3), 222.5 (+1.3), 246.5 (+13.1), 287 (+0.2). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 568 (70, M^+), 550 (100, $[M-H_2O]^+$), 532 (2, $[M-2\ H_2O]^+$), 458 (5), 388 (3), 263 (4), 237 (6), 209 (16), 197 (13), 174 (19), 157 (20), 145 (28), 135 (20), 121 (22), 119 (29), 105 (17), 95 (16), 69 (12), 43 (24).

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