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DETERMINATION OF THE GEOMETRICAL CONFIGURATION OF NATURALLY OCCURRING MONO-*CIS*-LUTEIN EPOXIDES

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Key Word Index—*Helianthus annuus*; Compositae; 9-, 9′-, 13-, and 13′-mono-*cis*-lutein epoxides; mono-*cis*-xanthophyll epoxides; geometrical configuration.

Abstract—By high-field two-dimensional ^1H NMR methods, the stereochemistry of the polyene chain has been assigned to four naturally occurring, isomeric mono-*cis*-lutein epoxides. The assignments corroborate our earlier suggestions according to which in *Helianthus annuus* the 9-*cis* isomer occurs without its 9′-*cis* counterpart. The stereomutation of natural lutein epoxide has also been studied.

INTRODUCTION

In an earlier paper dealing with the natural occurrence of the mono-*cis*-isomeric forms of some asymmetric C_{40}-carotenoids [1], we noted the presence of 9-, 9′- and 13(13′)-mono-*cis*-lutein epoxides in the blossoms of *Calendula officinalis*, *Chrysanthemum indicum* and *Helianthus annuus*. However, owing to incomplete separation of the isomers and the methods of their stereochemical assignments used at the time, the position of a *cis*-configured double bond within the polyene chain of individual natural pigments remained undefined. By improved chromatographic methods, four isomeric (3*S*, 5*R*, 6*S*, 3′*R*, 6′*R*)-mono-*cis*-lutein epoxides have now been separated in pure form and their respective stereochemistries unambiguously assigned by means of two-dimensional (2D) NMR techniques.

RESULTS AND DISCUSSION

The isolated natural lutein epoxide isomers **1–5** were separated by repeated column chromatography (see Experimental) and identified on the basis of their UV/Vis spectra. In view of the small quantities of the natural mono-*cis* isomers in the plant extracts, their comprehensive chemical and physico-chemical characterization was more conveniently performed on semisynthetic isomers prepared in larger amounts *via* iodine-catalysed photoisomerization of the more abundant naturally occurring all-*trans* lutein epoxide [(3*S*, 5*R*, 6*S*, 3′*R*, 6′*R*)-5,6-epoxy-5,6-dihydro- *β*,*ε*-carotene-3,3′-diol] (**1**). Column chromatography of the reaction mixture afforded the isomeric neolutein epoxide A′ (**2**), neolutein epoxide A″ (**3**), neolutein epoxide B′ (**4**) neolutein epoxide B″ (**5**), named in the order of their relative adsorption affinities [2, 3]. Correspondence between the natural and the semisynthetic mono-*cis* isomers (**2–5**) was unambiguously attested by co-chromatography and UV/Vis spectral data. It is noted that central-mono-*cis*-lutein epoxide ($\lambda_{max}^{C_6H_6}$ nm: 478, 458, 423 and 336; Q = 1.54; λ_{max}^{hexane} nm: 468, 438, 414 and 328; Q = 1.52; $\lambda_{max}^{C_6H_6}$ nm (after acid treatment): 458, 430, 407 and 321) was also detected, in traces, in the iodine catalysed stereoisomeric lutein epoxide mixture (Table 1).

The configurations of the double bonds in **2–5** were inferred from the 400 MHz ^1H NMR spectra and, as usual, based on the values of the observed interproton couplings and the chemical shift differences $\Delta = \delta_{cis} - \delta_{all-trans}$ measured for the olefinic protons in **2–5** and the all-*trans* reference molecule (**1**) [4]. Assignment of the resonance signals in terms of chemical shifts and proton–proton couplings was performed by means of standard 2D correlation spectroscopic (COSY) techniques [5, 6]. The fully assigned spectral parameters for isomers

Table 1. Composition of equilibrium mixture obtained from all-*trans*-lutein epoxide (**1**)

Percentage of isomers in the pigments recovered					
All-*trans*	Neo-A′	Neo-A″	Neo-B′	Neo-B″	Di-*cis*
(a) By refluxing in C_6H_6 soln in the dark					
72.1	13.1	9.9	—	—	4.9
(b) By iodine-catalysed stereomutation in light*					
56.1	7.2	5.4	11.1	11.9	8.4

*Traces of central-mono-*cis*-lutein epoxide were also detected.

1–5, together with relevant isomerization shift data, Δ, are collected in Table 2.

Corroborative chemical evidence for the correctness of the geometrical assignments of isomers **4** and **5** were readily available from the electronic spectra of the respective epimeric furanoid oxide derivatives [1, 7]: acid ($HCl–C_6H_6$) treatment of **5** resulted in epimeric furanoid oxides (flavoxanthin/chrysanthemaxanthin) with a λ_{max} characteristic of all-*trans* polyene chromophore (**6**), while isomer **4**, under similar conditions, gave mono-*cis* epimers exhibiting 5 nm hypsochromic shifts of their λ_{max}. These findings clearly showed that isomer **5** had its *cis*-configured double bond in position $\Delta^{9,10}$ and isomer **4** had it in $\Delta^{9',10'}$.

On the basis of similar arguments in our original paper [1] we noted that the co-occurrence of the various mono-*cis*-lutein epoxides may differ from plant to plant. The conclusive stereochemical assignments reported in this paper and a re-examination of the presence of isomeric mono-*cis*-lutein epoxides in *Helianthus annuus* have lent support to our earlier suggestions according to which the 9-*cis*-lutein epoxide isomer occurs in *H. annuus* together with the 13- and 13′-mono-*cis* forms without any detectable amounts of the 9′-*cis* isomer

EXPERIMENTAL

General methods, including routine instrumentation and CC ($CaCO_3$; Biogal, Hungary) have been described elsewhere [6]. The pigments on the chromatograms are listed in order of decreasing adsorption affinities.

Conventional and homonuclear proton chemical shift correlated 2D spectra were obtained at 400 MHz using standard software. In order to prevent cleavage of the highly sensitive epoxide rings during measurements, all NMR spectra were run on pyridine-d_5 solns (1.5 mg in 0.6 ml) at ambient temp.

Plant material. H. annuus (Pécs, 1986; 21.4 g fr. wt). Pigments in petals: 13-*cis*-violaxanthin, violeoxanthin, 13(13′)-*cis*-lutein epoxide(s), 9-*cis*-lutein epoxide, 9(9′)-*cis*-lutein(s) (trace), violaxanthin, lutein epoxide and lutein. The zones above 9-*cis*-lutein

Table 2. ^1H NMR data of isomeric lutein epoxides*

H	all-*trans* (**1**)	13′-*cis* (**2**)		13-*cis* (**3**)		9′-*cis* (**4**)		9-*cis* (**5**)	
	δ_H	δ_H	Δ	δ_H	Δ	δ_H	Δ	δ_H	Δ
7	6.161	6.148	−0.01	6.178	0.02	6.153	−0.01	6.230	0.07
8	6.661	6.652	−0.01	6.673	0.01	6.665	0.00	7.319	0.66
10	6.348	6.333	−0.02	6.396	0.05	6.346	0.00	6.271	−0.08
11	6.855	6.842	−0.01	6.851	0.00	6.847	−0.01	7.062	0.21
12	6.598	6.588	−0.01	7.208	0.61	6.592	−0.01	6.522	−0.08
14	6.478	6.480	0.00	6.274	−0.20	6.476	0.00	6.439	−0.04
15	6.835	6.763	−0.07	7.174	0.34	6.818	−0.02	6.763	−0.07
Me-16	1.265	1.261	0.00	1.266	0.00	1.263	0.00	1.262	0.00
Me-17	1.150	1.146	0.00	1.144	−0.01	1.148	0.00	1.137	−0.01
Me-18	1.286	1.281	0.00	1.284	0.00	1.283	0.00	1.277	−0.01
Me-19	2.027	2.019	−0.01	2.022	−0.01	2.022	−0.01	2.045	0.02
Me-20	2.010	2.008	0.00	2.031	0.02	2.002	−0.01	1.829	−0.18
7′	5.654	5.677	0.02	5.644	−0.01	5.701	0.05	5.655	0.00
8′	6.333	6.378	0.05	6.336	0.00	7.101	0.77	6.332	0.00
10′	6.382	6.465	0.08	6.369	−0.01	6.236	−0.15	6.373	−0.01
11′	6.878	6.879	0.00	6.857	−0.02	7.205	0.33	6.865	−0.01
12′	6.624	7.250	0.63	6.617	−0.01	6.551	−0.07	6.629	0.01
14′	6.477	6.267	−0.21	6.516	0.04	6.471	−0.01	6.463	−0.01
15′	6.834	7.135	0.30	6.777	−0.06	6.814	−0.02	6.804	−0.03
Me-16′	0.953	0.951	0.00	0.949	0.00	0.942	−0.01	0.951	0.00
Me-17′	1.134	1.129	−0.01	1.128	−0.01	1.102	−0.03	1.132	0.00
Me-18′	1.711	1.707	0.00	1.706	0.00	1.699	−0.01	1.710	0.00
Me-19′	1.993	1.994	0.00	1.984	−0.01	2.009	0.02	1.987	−0.01
Me-20′	2.027	2.044	0.02	2.022	−0.01	2.022	−0.01	2.006	−0.02
$J_{7,8}$	15.7	15.6		15.6		15.5		15.4	
$J_{10,11}$	11.7	11.7		11.9		11.6		11.4	
$J_{11,12}$	15.0	14.9		14.8		15.1		15.0	
$J_{14,15}$	11.0	11.8		11.9		11.0		11.0	
$J_{15,15'}$	14.5	14.2		14.3		14.6		14.5	
$J_{7',8'}$	15.4	15.4		15.4		15.3		15.4	
$J_{10',11'}$	11.2	11.4		11.5		11.4		11.3	
$J_{11',12'}$	14.9	14.9		14.9		15.0		15.0	
$J_{14',15'}$	11.0	11.7		11.8		11.0		11.0	

Head-group protons: H-2_{ax} 1.59, H-2_{eq} 1.91, H-3 4.29, H-4_{ax} 2.00, H-4_{eq} 2.64, H-$2'_{ax}$ 1.73, H-$2'_{eq}$ 2.05, H-3′ 4.61, H-4′ 5.94, H-6′ 2.54; $J_{2_{ax},2_{eq}} = -12.7$, $J_{2_{ax},3} = 10.3$, $J_{2_{eq},3} = 3.4$, $J_{2_{eq},4_{eq}} = 1.5$, $J_{3,4_{ax}} = 8.6$, $J_{3,4_{eq}} = 4.9$, $J_{4_{ax},4_{eq}} = -14.4$, $J_{2'_{ax},2'_{eq}} = -12.9$, $J_{2'_{ax},3'} = 7.3$, $J_{2'_{eq},3'} = 5.9$, $J_{3',4'} < 0.5$, $J_{6',7'} = 9.8$. These resonances show minor ($\leqslant 0.01$ ppm) upfield shifts upon *trans–cis* isomerization at the 9(9′)-double bond. $\Delta = \delta_{cis} - \delta_{\text{all-}trans}$.

*In pyridine-d_5 soln, at 20°. Chemical shifts are in ppm relative to internal TMS, coupling constants in Hz.

epoxide after the usual work-up were combined and subjected to re-chromatography on deactivated $CaCO_3$ with C_6H_6 containing Me_2CO gradually increased up to 0.1%. The following zones were obtained: band 1 (13-*cis*-violaxanthin), band 2 (9-*cis*-violaxanthin), band 3 (neolutein epoxide A′ and A″) and band 4 [luteoxanthin epimer(s), traces]. The sepn of the constituents of band 3 was achieved on deactivated $CaCO_3$ with a mixt. of C_6H_6 satd with H_2O and petrol (9:1). Neolutein epoxide A′ and A″ obtained in *ca* the same amount have physical and spectral properties similar to those of authentic samples.

Neolutein epoxide A′ was inseparable from authentic 13′-*cis*-lutein epoxide (**2**) by co-chromatogram (deactivated $CaCO_3$), but it sepd from its counterpart (**3**). UV/Vis $\lambda_{\max}^{C_6H_6}$ nm: 476, 446, 420 and 337; $Q = 1.92$.

Neolutein epoxide A″ was inseparable from authentic 13-*cis*-lutein epoxide by co-chromatogram as above, but it sepd from its counterpart (**2**). UV/Vis $\lambda_{\max}^{C_6H_6}$ nm: 475, 445, 420 and 337; $Q = 2.07$. Similar co-chromatography proved in agreement with our earlier finding that 9-*cis*-lutein epoxide (**5**) was present as a single peripheral isomer without its 9′-*cis* counterpart.

Preparation of authentic isomers (**2–5**). *Iodine catalysed stereomutation of all*-trans-*lutein epoxide* (**1**) *in light*. A mixt of 9-*cis*-, 9′-*cis*-, 13-*cis*- and 13′-*cis*-lutein epoxide were prepd from 180 mg (3*S*, 5*R*, 6*S*, 3′*R*′, 6′*R*)-lutein epoxide (**1**) (ex. *H. annuus* and *T. officinale*) by I_2 catalysis in diffuse daylight [8]. Chromatography on $CaCO_3$ with C_6H_6 gave three main zones: band 1 (**2, 3**), band 2 (**4, 5**) and band 3 (**1**).

Sepn of the constituents of band 1 (**2, 3**) was achieved on $CaCO_3$ with C_6H_6 satd with H_2O. The following zones were obtained: band 11 (**2**), band 12 (**3**) and several narrow unidentified bands. A mixt. of Et_2O and petrol (30–40°) was used for crystallization.

Neolutein epoxide A′ (**2**) (*band* 11). Dark red, irregular forms (7.8 mg) were pptd, mp 88°. UV/Vis $\lambda_{\max}^{C_6H_6}$ nm (log ε): 476 (4.85), 446 (4.92), 420 (4.78) and 337 (4.64); $Q = 1.93$; $\lambda_{\max}^{\text{hexane}}$ nm: 463, 434 and 410; $\lambda_{\max}^{\text{petrol}}$ nm: 464, 435 and 411; $\lambda_{\max}^{\text{EtOH}}$ nm: 464, 435 and 412. $\lambda_{\max}^{C_6H_6}$ (after acid treatment) nm: 456, 428 and 404.

Neolutein epoxide A″ (**3**) (*band* 12). Dark purple, amorphous (3.7 mg), mp 84°. UV/Vis $\lambda_{\max}^{C_6H_6}$ (log ε) nm: 4.75 (4.77), 445 (4.83), 419 (4.66) and 337 (4.54); $Q = 2.08$; $\lambda_{\max}^{\text{hexane}}$ nm: 462, 432 and 409; $\lambda_{\max}^{\text{petrol}}$ nm: 463, 433 and 409; $\lambda_{\max}^{\text{EtOH}}$ nm: 463, 434 and 411. $\lambda_{\max}^{C_6H_6}$ (after acid treatment) nm: 456, 427 and 404.

Re-chromatography of band 2 (**4, 5**) on $CaCO_3$ with a C_6H_6–petrol (40–60°) (9:1) satd with H_2O gave band 21 (**4**), band 22 (**5**) and several narrow unidentified bands. The isomers were pptd from Et_2O with petrol (30–40°).

Neolutein epoxide B′ (**4**) (*band* 21). Orange red, amorphous (23 mg), mp 90°. Vis $\lambda_{\max}^{C_6H_6}$ (log ε) nm: 478 (4.98), 448 (5.01) and 423 (4.89); $\lambda_{\max}^{\text{hexane}}$ nm: 464, 434 and 412; $\lambda_{\max}^{\text{petrol}}$ nm: 466, 436 and 413; $\lambda_{\max}^{\text{EtOH}}$ nm: 465, 436 and 414. $\lambda_{\max}^{C_6H_6}$ (after acid treatment) nm: 455, 426 and 403.

Neolutein epoxide B″ (**5**) (*band* 22). Orange-red, amorphous (25.4 mg), mp 102°. Vis $\lambda_{\max}^{C_6H_6}$ (log ε) nm: 478 (5.02), 448 (5.06) and 423 (4.94); $\lambda_{\max}^{\text{hexane}}$ nm: 464, 434 and 412; $\lambda_{\max}^{\text{petrol}}$ nm: 465, 435 and 412; $\lambda_{\max}^{\text{EtOH}}$ nm: 465, 436 and 414. $\lambda_{\max}^{C_6H_6}$ (after acid treatment) nm: 459, 430 and 406.

Natural neolutein epoxide B″ (**5**) (ex. *H. annuus*). Mp 97°; Vis $\lambda_{\max}^{C_6H_6}$ (log ε) nm; 477 (5.07), 447 (5.09) and 421 (4.93); $\lambda_{\max}^{\text{hexane}}$ nm: 463, 433 and 411; $\lambda_{\max}^{\text{petrol}}$ nm: 464, 434 and 411; $\lambda_{\max}^{\text{EtOH}}$ nm: 464, 435 and 413; $\lambda_{\max}^{C_6H_6}$ (after acid treatment) nm: 459, 430 and 406.

Reference compound. (3*S*, 5*R*, 6*S*, 3′*R*, 6′*R*)-lutein epoxide (**1**) (ex. *H. annuus*). Mp 156°; Vis $\lambda_{\max}^{C_6H_6}$ (log ε) nm: 483 (5.08), 452 (5.10) and 427 (4.93); $\lambda_{\max}^{\text{hexane}}$ nm: 471, 440 and 418; $\lambda_{\max}^{\text{petrol}}$ nm: 471, 441 and 418; $\lambda_{\max}^{\text{EtOH}}$ (log ε) nm: 471 (5.15), 443 (5.17) and 418 (4.99). $\lambda_{\max}^{C_6H_6}$ (after acid treatment) nm: 459, 430 and 406.

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