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OCCURRENCE OF SOME MONO-*CIS*-ISOMERS OF ASYMMETRIC C_{40}-CAROTENOIDS

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Key Word Index—*Lilium candidum*; Liliaceae; *Calendula officinalis*; *Helianthus annuus*; *Helianthus debilis*; *Chrysanthemum indicum*; Compositae; *Citrus aurantium*; Rutaceae; 9-*cis*-antheraxanthin; 9-*cis*-lutein epoxide.

Abstract—The 9-*cis*-isomers of antheraxanthin [(3*S*,5*R*,6*S*)-5,6-epoxy-5,6-dihydro-*β*, *β*-carotene-3,3′-diol] and lutein epoxide [(3*S*,5*R*,6*S*,3′*R*,6′*R*)-5,6-epoxy-5,6-dihydro-*β*, *ε*-carotene-3,3′-diol] were found to occur without their 9′-*cis* counterparts in the non-photosynthetic tissues of several higher plants. 9-*cis*-lutein [(3*R*,3′*R*,6′*R*)-*β*,*ε*-carotene-3,3′-diol], on the other hand, was observed together with its 9′-*cis* counterpart in the samples investigated. The qualitative distribution of carotenoids is also reported.

INTRODUCTION

Iodine-catalysed stereomutation of constitutionally asymmetric C_{40}-carotenoids was found to give four mono-*cis*-isomers [1], which was ascribed to the non-equivalence of positions C-9 and C-9′, C-13 and C-13′ (excluding the central mono-*cis* form). ^{13}C NMR spectroscopy had provided unambiguous stereochemical assignment for the isolated mono-*cis*-isomers ([2]; Molnár, P., Szabolcs, J. and Radics, L., unpublished results). Thus on the basis of the existence, in equilibrium mixtures, of the 9-, 9′-, 13-, 13′-*cis*-isomers of antheraxanthin [(3*S*,5*R*,6*S*,3′*R*)-5,6-epoxy-5,6-dihydro-*β*,*β*-carotene-3,3′-diol], capsanthin [(3*R*,3′*S*,5′*R*)-3,3′-dihydroxy-*β*,*κ*-carotene-6′-one], lutein [(3*R*,3′*R*,6′*R*)-*β*,*ε*-carotene-3,3′-diol] and lutein epoxide [(3*S*,5*R*,6*S*,3′*R*,6′*R*)-5,6-epoxy-5,6-dihydro-*β*,*ε*-carotene-3,3′-diol], we have made a systematic search to prove their existence or non-existence in nature. Although there have been many reports [3–9] on the occurrence of mono-*cis*-isomers derived from asymmetric all-*trans* compounds, it has never been clarified whether 9-*cis* or 9′-*cis* or both, 13-*cis* or 13′-*cis* or both isomers are present in the sample investigated.

RESULTS AND DISCUSSION

As the mono-*cis*-isomers of the asymmetric C_{40}-carotenoids at our disposal belong to the higher oxidized pigments and the amount of the naturally occurring next-to-central isomers (13/13′-*cis*) is too small for further separation, our study was limited to non-photosynthetic tissues of higher plants, on the one hand, and to the occurrence of only the peripheral (9/9′-*cis*)-isomers on the other.

Tables 1 and 2 show the distribution of the mono-*cis*-isomers and the all-*trans* forms in anthers, petals and fruits, and for comparison, the compositions of the iodine-catalysed equilibrium mixture of the corresponding carotenoid pigment. The analyses have revealed the following:

(a) 9-*cis*-antheraxanthin (**2**) always occurs as a single peripheral isomer. Its 9′-*cis* counterpart (**1**) has not been detected;
(b) 9-*cis*-lutein epoxide (**4**) is present, as a single peripheral isomer in all cases except one in which its 9′-*cis* counterpart (**3**) is preponderant;
(c) 9-*cis* antheraxanthin (**2**) occurs together with 9-*cis*-lutein epoxide (**4**) without their 9′-*cis* counterparts (**1**, **3**) in *Calendula*;
(d) in general, 9-*cis* epoxides occur in larger quantity than the corresponding all-*trans* forms;
(e) 9-*cis*-lutein (**6**) and 9′-*cis*-lutein (**5**) always appear together in about the same percentage;
(f) the proportions of 9-*cis*- and 9′-*cis*-isomers in native samples are different from those in the equilibrium mixtures obtained by iodine catalysis.

Considering the high percentages of 9′-*cis* epoxides (**2**, **4**) as compared with the corresponding all-*trans* forms, and the very mild conditions under which all operations were carried out, it seems doubtless that 9-*cis*-antheraxanthin (**2**) and 9-*cis*-lutein epoxide (**4**) are authentic natural products. In both molecules the *cis*-double bond is located near the 5,6-epoxy end (3*S*,5*R*,6*S*) of the molecule. This is in good agreement with the preponderant occurrence of 9-*cis*-violaxanthin (**9**) [(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-*β*,*β*-carotene-3,3′-diol] in flowers of *Viola tricolor* (60–70%) [10] and with the fact that 9-*cis*-zeaxanthin (**10**) [(3*R*,3′*R*)-3,3′-dihydroxy-*β*,*β*-carotene] is very rare in nature. Similarly, neoxanthin (**11**) [(3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-9′-*cis*-5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-*β*,*β*-carotene-3,5,3′-triol], whose 9-*cis* counterpart cannot exist because of the peripheral allenic bond, is very common in nature [11, 12]. All these observations demonstrate the stability of the 9-*cis* type configuration of epoxy carotenoids as compared with that of non-epoxy carotenoids in nature.

The individual appearance of 9-*cis*-antheraxanthin (**2**) and 9-*cis*-lutein epoxide (**4**) might be explained in one of two ways or by a combination of the two: (i) They are formed by stereoselective biosynthesis or by stereo-

Table 1. Quantitative distribution of the individual isomers in a representative group of higher plants (expressed as percentage of total pigment recovered)

Pigments	*Lilium candidum*	*Calendula officinalis* (yellow)	*Calendula officinalis* (orange)	*Helianthus annuus*	*Helianthus debilis*	*Chrysanthemum indicum* 'Champion'	*Citrus aurantium*	*Capsicum annuum* (red)
Antheraxanthin								
9-*cis*	55.0	4.0	4.3	—	14.0	—	13.6	—
9′-*cis*	—	—	—	—	—	—	—	—
all-*trans*	6.8	—	—	—	4.2	—	1.1	—
Lutein epoxide								
9-*cis*	—	8.3	4.1	25.7	—	10.7	—	—
9′*cis*	—	—	—	—	—	43.8	—	—
all-*trans*	—	7.2	6.2	27.6	—	6.6	—	—
Lutein								
9-*cis*	—	4.3	2.3	+	1.3	9.6	—	—
9′-*cis*	—	3.2	2.2	+	1.2	3.8	—	—
all-*trans*	—	8.6	4.6	20.4	55.4	4.2	3.6	—
Capsanthin								
9-*cis*	—	—	—	—	—	—	—	0.7
9′-*cis*	—	—	—	—	—	—	—	?
all-*trans*	—	—	—	—	—	—	—	33.2

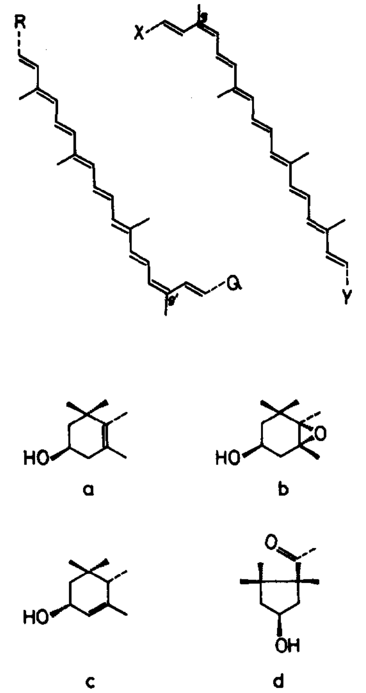
Table 2. Quantitative distribution of 9-*cis*-, 9′-*cis*- and all-*trans*-stereoisomers in a representative group of higher plants (expressed as percentage of a certain stereoisomeric set*)

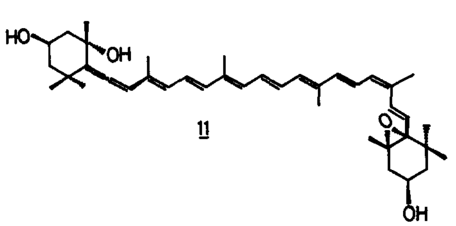
Pigments	*Lilium candidum*	*Calendula officinalis* (yellow)	*Calendula officinalis* (orange)	*Helianthus annus*	*Helianthus debilis*	*Chyrsanthemum indicum* 'Champion'	*Citrus aurantium*	Equilibrium mixture izomerized by iodine‡
Antheraxanthin								
9-*cis*	77.8	100.0	100.0	—	76.9	—	92.5	12.8
9′-*cis*	—	—	—	—	—	—	—	11.9
all-*trans*	9.6	—	—	—	23.1	—	7.5	51.3
Lutein epoxide								
9-*cis*	—	53.5	39.8	43.2	—	15.5	—	10.9
9′*cis*	—	—	—	—	—	63.4	—	8.9
all-*trans*	—	46.5	60.2	46.4	—	9.6	—	56.6
Lutein								
9-*cis*	—	26.7	25.3	+	2.2	54.5	—	12.0
9′-*cis*	—	19.9	24.2	+	2.0	21.6	—	11.0
all-*trans*	—	53.4	50.5	96.7	93.9	23.9	100.0	60.0

* Including the total *cis–trans* isomeric forms observed.

† In traces.

‡ For comparison.





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1 R = b Q = a
2 X = b Y = a
3 R = b Q = c
4 X = b Y = c
5 R = a Q = c
6 X = a Y = c
7 X = a Y = d
8 R = a Q = d
9 X = Y = b
10 X = Y = a

selective stereomutation from the all-*trans* forms, and after they have been formed their configuration is protected against photo-induced stereomutation. (ii) Both counterparts are formed by non-stereoselective biosynthesis or stereomutation, but only the 9-*cis* form is protected stereoselectively against photoisomerization.

EXPERIMENTAL

Methods. The general methods, including instrumentation, zone chromatography, identification and quantitative determination of the individual carotenoids have been described elsewhere [13]. All operations were performed in darkness. The pigments on the chromatograms are listed in the order of decreasing adsorption affinities.

Preparation of the authentic key-isomers (**1**,**2**,**5**–**8**) is described elsewhere [2].

Pigment isolation. The samples (4–10 g) were collected in MeOH and kept for dehydration at 4–6° in darkness for 24 hr. Then the methanolic solns were filtered and the materials were homogenized with cold MeOH in an electric blender. The extractions were completed with Et_2O, and the combined MeOH and Et_2O extracts were washed with H_2O, dried (Na_2SO_4) and saponified with 30% methanolic KOH for 12 hr. Finally, the Et_2O solns were washed free from alkali with H_2O, dried (Na_2SO_4), evapd to dryness *in vacuo*, dissolved in C_6H_6, and chromatographed on $CaCO_3$ (Biogal, Hungary) with a C_6H_6–petrol (40–60°) mixture (4:1). In several cases, the zones of 9(9′)-mono-*cis*-isomers were subjected to re-chromatographies on $CaCO_3$ with C_6H_6–Me_2CO (100:1) for perfect sepn.

Biological materials. Lilium candidum (Pécs, 1976). Pigments in anthers: violeoxanthin, 9-*cis*-antheraxanthin (**2**), di*cis*-violaxanthin, violaxanthin, di*cis*-antheraxanthins, antheraxanthin and lutein.

Starting with 45 g (dry wt) of anthers, 30 mg of **2** were obtained. It crystallized from MeOH; mp 115°; UV $\lambda_{max}^{C_6H_6}$ nm (log ε): 483 (4.99), 453 (5.05) and 430 (4.88). The pigment was inseparable from authentic **2** on a mixed chromatogram [$CaCO_3$, C_6H_6–Me_2CO(100:1)], but it separated from its authentic counterpart (**1**). A furanoid oxide test [6] of **2** gave 85% of all-*trans* mutatoxanthin epimers and 15% of mono-*cis* epimers ($\lambda_{max}^{C_6H_6}$ shifts are 5 nm). For comparison, **1** yielded 100% of mono-*cis* mutatoxanthins under the same conditions.

Citrus aurantium (*El-Asnam d'Algire*, 1978). Pigments in pulp: an unidentified minor pigment, 13-*cis*-violaxanthin, violeoxanthin, *cis*-luteoxanthin, 9-*cis*-antheraxanthin (**2**), luteoxanthin epimers, a mixture of *cis*-zeaxanthin and *cis*-luteoxanthin, mutatoxanthin, luteoxanthin epimer, violaxanthin, antheraxanthin, zeaxanthin, lutein and β-carotene.

Calendula officinalis (*yellow*) (*Pécs*, 1979). Pigments in petals: violeoxanthin, auroxanthin, *cis*-luteoxanthin, 9-*cis*-lutein epoxide (**4**), luteoxanthin, *cis*-flavoxanthin, 9′-*cis*-lutein (**5**), 9-*cis*-lutein (**6**), flavoxanthin, lutein epoxide, lutein and β-carotene.

Calendula officinalis (*orange*) (*Pécs*, 1979). Pigments in petals: violeoxanthin, auroxanthin, *cis*-luteoxanthin, 9-*cis*-antheraxanthin (**2**), 9-*cis*-lutein epoxide (**4**), luteoxanthin, an unidentified minor pigment, 9′-*cis*-lutein (**5**), 9-*cis*-lutein (**6**), mutatoxanthin, flavoxanthin, lutein epoxide, lutein, lycopene and β-carotene.

Helianthus annuus (*Pécs*, 1978). Pigments in petals: 13-*cis*-violaxanthin, violeoxanthin, mixture of 13-*cis*- and 13′-*cis*-lutein epoxide, 9-*cis*-lutein epoxide (**4**), 9(9′)-*cis*-lutein(s) (in traces), violaxanthin, lutein epoxide and lutein.

Petals (20 g dry wt) were worked up on a preparative scale yielding 25 mg of **4**. It crystallized from a mixture of C_6H_6 and petrol (30–40°); mp 88°; UV $\lambda_{max}^{C_6H_6}$ nm (log ε): 478 (4.92), 448 (4.93) and 424 (4.74). The pigment was inseparable from authentic **4** on a mixed chromatogram [$CaCO_3$, C_6H_6–Me_2CO (100:1)], but it separated from its counterpart (**3**). A furanoid oxide test [6] of **4** resulted in 87% of all-*trans* furanoid oxide epimers (chrysanthemaxanthin and flavoxanthin) and 13% of mono-*cis* furanoid oxides ($\lambda_{max}^{C_6H_6}$ shifts are 4 nm). For comparison, **3** gave 14% of all-*trans* and 86% of mono-*cis* furanoid oxide under the same conditions.

Helianthus debilis (*Pécs*, 1978). Pigments in petals: 13-*cis*-violaxanthin, violeoxanthin, 9-*cis*-antheraxanthin (**2**), 13(13′)-*cis*-lutein(s), 9′-*cis*-lutein (**5**), 9-*cis*-lutein (**6**), di-*cis*-violaxanthin, antheraxanthin and lutein.

Chrysanthemum indicum '*Champion*' (*vicinity of Pécs*, 1980). Pigments in petals: an unidentified pigment (in traces), violeoxanthin, 13(13′)-*cis*-lutein epoxide(s), 9′-*cis*-lutein epoxide (**3**), 9-*cis*-lutein epoxide (**4**), 9′-*cis*-lutein (**5**), 9-*cis*-lutein (**6**), di-*cis*-lutein epoxides (?), lutein epoxide, lutein and an unidentified pigment with epiphasic properties.

Viola tricolor (*Pécs*, 1978). Pigments in petals: 13-*cis*-violaxanthin, violeoxanthin, 9-*cis*-antheraxanthin (**2**), luteoxanthin, di-*cis*-violaxanthins, violaxanthin, antheraxanthin, lutein and β-carotene.

Capsicum annuum lycopersiciforme rubrum (*Pécs*, 1980). Pigments (adsorbed above capsorubin) in fruits: *cis*-capsorubins, 13-*cis*-capsanthin, 13′-*cis*-capsanthin, 9-*cis*-capsanthin (**7**), 9′-*cis*-

capsanthin (**8**), capsorubin, etc. It is noted that 9′-*cis*-capsanthin (**8**), as a result of mixed chromatography with authentic **7**, adsorbs between **7** and capsorubin.

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