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Carotenoid composition in petals of chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura)

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Abstract

Sixteen xanthophylls were isolated from the petals of chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura). Among them, (3*S*,5*S*,6*R*,3′*R*,6′*R*)-5,6-dihydro-5,6-dihydroxylutein (**1**) and five di-*Z* geometrical isomers of lutein-5,6-epoxide, i.e., 9*Z*,13′*Z* (**2**), 13*Z*,9′*Z* (**3**), 9′*Z*,13′*Z* (**4**), 9*Z*,13*Z* (**5**), and 9*Z*,9′*Z* (**7**), had never before been identified as natural products. All of the carotenoids isolated from chrysanthemum, except for (9*Z*)-violaxanthin, are β,ε-carotene (α-carotene) derivatives. The analyses indicate that carotenoids from the petals of chrysanthemum have a very characteristic composition.

Keywords: *Dendranthema grandiflorum* (Ramat.) Kitamura; Compositae; Chrysanthemum; NMR; Petals; Carotenoids; 5,6-Dihydro-5,6-dihydroxylutein; (di-*Z*)-Lutein-5,6-epoxide

1. Introduction

Flowers owe their colors to flavones, anthocyanins, or carotenoids. Carotenoids are usually responsible for petal colors in the yellow-to-orange range. Several investigations of the carotenoid compositions of petals have been reported so far (Bakó et al., 2002; Deli et al., 1988, 1998a, 2000; Eugster and Märki-Fischer, 1991; Goodwin and Britton, 1988; Khachik et al., 1999; Kull and Pfander, 1995, 1997; Maoka et al., 2000; Tai and Chen, 2000; Zhu et al., 2003). In general, the leaves of most plants show similar carotenoid compositions, containing both β,ε-carotene (α-carotene) derivatives and β,β-carotene (β-carotene) derivatives (Goodwin and Britton, 1988). In contrast, carotenoids in petals show some distinctive compositions that depend on the plant species. For example, petals of *Lilium lancifolium* contain only β-carotene derivatives (Deli et al., 1998a, 2000) and those of *Tagetes erecta* contain a large amount of lutein, an α-carotene derivative (~91% of their total carotenoids) (Khachik et al., 1999). The carotenoid compositions of four Compositae species, including *T. erecta*, have been reported so far, and all have a tendency to accumulate lutein and/or lutein derivatives as the major carotenoid components (Bakó et al., 2002; Deli et al., 1988; Tóth and Szabolcs, 1981).

Chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) is one of the most important ornamental plants in the world, and the color of the petals of yellow varieties mainly originates from carotenoid pigments. Some carotenoids have been characterized by TLC and absorption spectroscopy (Karrer and Jucker, 1943; Karrer et al., 1945; Kawase and Tsukamoto, 1976); however, accurate molecular structures were not determined. Tóth and Szabolcs (1981) characterized eight carotenoids, including five mono-*Z*-isomers, using

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synthetic standard preparations, but they were unable to identify several unknown components.

Here, we report the results of a detailed investigation that showed that chrysanthemum petals have a unique carotenoid composition. In addition, we identified six carotenoids, including (3*S*,5*S*,6*R*,3′*R*,6′*R*)-5,6-dihydro-5,6-dihydroxylutein (**1**) and five di-*Z* geometrical isomers of lutein-5,6-epoxide (**2**–**5**, **7**), as natural products for the first time.

2. Results and discussion

MeOH solutions of mature petals of 12 chrysanthemum cultivars which showed yellow-to-red range were analyzed by HPLC. They showed similar HPLC chromatographs; here we indicate that of cv. 'Sunny Orange' as a representative (Fig. 1). Thirteen fractions were obtained by preparative HPLC on a C30 column, and peaks 2 and 4 were further purified by HPLC on a silica gel column (see Table 1).

Compound **1** (from peak 1 in Fig. 1 ; structure in Fig. 2) showed absorption maxima at wavelengths of 414, 438, and 467 nm, similar to those of lutein (Britton, 1995). High-resolution FABMS established the molecular formula as $C_{40}H_{56}O_4$. The ^{13}C and ^{1}H NMR data for **1**, as assigned by ^{1}H–^{1}H COSY, NOESY, HSQC and HMBC experiments, are shown in Table 2. The presence of two secondary hydroxy groups (δ_C 66.3, δ_H 3.97 and δ_C 65.9, δ_H 4.25) and two tertiary hydroxy groups (δ_C 79.4 and 76.3) was revealed by analysis of the ^{13}C and ^{1}H NMR spectral data. The partial structures of the 3-hydroxy-ε-end group and the all-*Z* polyene chain of **1** were confirmed by analysis of the ^{13}C and ^{1}H NMR spectral data in comparison with that for lutein (Englert, 1995). The partial structure of the other end group (the 3,5,6-trihydroxy-5,6-dihydro-β-end group) was elucidated as follows: the ^{1}H–^{1}H COSY and HSQC experiments established the proton and carbon connectivity from C-2 to C-4 and the position of a secondary hydroxy group at C-3 (δ_C 66.3, δ_H 3.97). The quaternary carbons at δ_C 38.7, 76.3 and 79.4 were assigned to be C-l, C-6, and C-5, respectively, by HMBC data. The locations of two tertiary hydroxy groups were deduced to be at C-5 and C-6. Three methyl groups were also assigned by HSQC and HMBC correlations, as described in the Section 3. NOESY correlation H-16/H-2α, H-16/H-17, H-18/H-3 and H18/H17 indicated that CH_3-16, CH_3-18, H-2α, H-3 and H-7 are located on the same side of this end group and consequently (3*S*,5*S*,6*R*)-configuration for **1** can be proposed (Fig. 2). We compared CD data of **1** with data of the synthetic compound (Buchecker et al., 1984) in order to determine the absolute configuration, although the data do not provide sufficient evidence of a (3*S*,5*S*,6*R*)-configuration of the 3,5,6-trihydroxy group. The ^{1}H and ^{13}C NMR spectrascope data of the 3,5,6-trihydroxy group in **1** do not coincide with those of (3*S*,5*R*,6*R*), (3*S*,5*R*,6*S*), (3*S*,5*S*,6*S*) and (3*S*,5*S*,6*R*)-carotenoids reported by Molnár et al. (1999) and Deli et al. (1998b) but coincide with (3*S*,5*S*,6*R*,3′*R*,6′*R*)-5,6-dihydro-β,ε-carotene-3,5,-6,3′-tetrol reported by Buchecker et al. (1984) and Eugster (1985). Thus, the structure of **1** was determined to be (3*S*,5*S*,6*R*,3′*R*,6′*R*)-5,6-dihydro-β,ε-carotene-3,5, 6,3′-tetrol [(3*S*,5*S*,6*R*,3′*R*,6′*R*)-5,6-dihydro-5,6-dihydroxylutein]. This compound was previously synthesized by Buchecker et al. (1984) and Eugster (1985). However, it had not yet been isolated from nature. This is the first report of the isolation and full characterization by ^{13}C and ^{1}H NMR of **1** as a natural product.

Synthetic compound **1** was derived from (3*S*,5*S*,6*R*,3′*R*,6′*R*)-lutein-5,6-epoxide by mild epoxide hydrolysis (Buchecker et al., 1984; Eugster, 1985; Fig. 2); however, (3*S*,5*S*,6*R*,3′*R*,6′*R*)-lutein-5,6-epoxide had never been isolated as a natural compound. It is possible that **1** is formed from (3*S*,5*R*,6*S*,3′*R*,6′*R*)-lutein-5,6-epoxide (**6**) by epoxide hydrolysis and sequential isomerization of C-5 and C-6 hydroxyl groups. Elucidation of the proposed biosynthetic pathway of **1** (Fig. 2) requires further investigation.

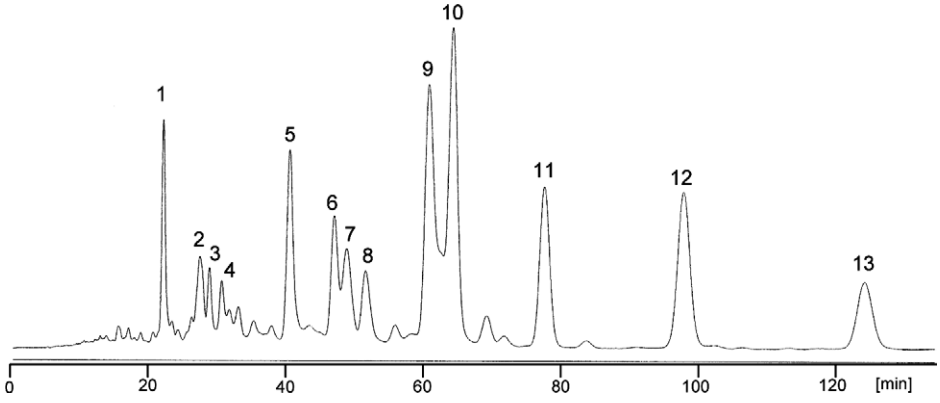

Fig. 1. HPLC separation of caratenoids of a extract of chrysanthenum petals (cv. 'Sunny Orange'). (Peak numbers as in Table 1.)

Table 1
Carotenoid composition in petals of chrysanthemum (cv. 'Sunny Orange')

Peak no. (Fig. 1)	Carotenoids	% of total carotenoids
1	(3*S*,5*S*,6*R*,3′*R*,6′*R*)-5,6-Dihydro-5,6-dihydroxylutein (**1**)	5.1
2	(9*Z*,13′*Z*)-Lutein-5,6-epoxide (**2**)	1.8
	(13*Z*,9′*Z*)-Lutein-5,6-epoxide (**3**)	1.8
3	(9′*Z*,13′*Z*)-Lutein-5,6-epoxide (**4**)	2.2
4	(9*Z*,13*Z*)-Lutein-5,6-epoxide (**5**)	2.0
5	(all-*E*)-Lutein-5,6-epoxide (**6**)	7.7
6	(9*Z*,9′*Z*)-Lutein-5,6-epoxide (**7**)	2.5
	(9*Z*)-Violaxanthin	2.7
7	(8*S*)-Lutein-5,8-epoxide (=chrysanthemaxanthin)	5.0
8	(8*R*)-Lutein-5,8-epoxide (=flavoxanthin)	1.7
	(9*Z*-8′*R*)-Luteoxanthin	1.8
9	(9′*Z*)-Lutein-5,6-epoxide (**8**)	16.6
10	(9*Z*)-Lutein-5,6-epoxide (**9**)	16.9
11	(all-*E*)-Lutein	9.4
12	(9*Z*)-Lutein	11.3
13	(9′*Z*)-Lutein	6.0

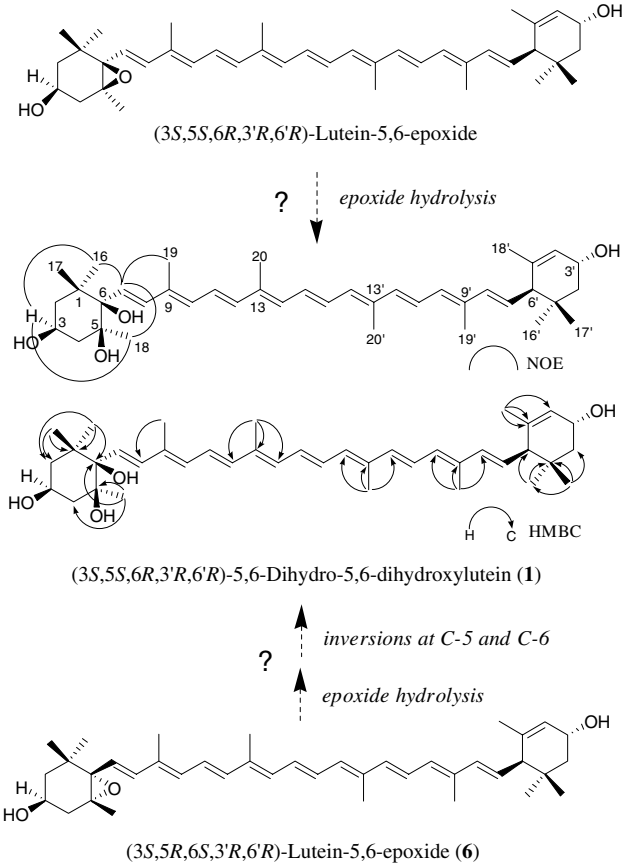

Fig. 2. Structure of (3*S*,5*S*,6*R*,3′*R*,6′*R*)-5,6-dihydro-5,6-dihydroxylutein (**1**) and putative biosynthetic pathway of **1**.

Eight geometrical isomers of lutein-5,6-epoxide, i.e., 9*Z*,13′*Z* (**2**, from peak 2), 13*Z*,9′*Z* (**3**, from peak 2), 9′*Z*,13′*Z* (**4**, from peak 3), 9*Z*,13*Z* (**5**, from peak 4), all-*E* (**6**, from peak 5), 9*Z*,9′*Z* (**7**, from peak 6), 9′*Z* (**8**, from peak 9), and 9*Z* (**9**, from peak 10) were isolated. Among them, five di-*Z* isomers are new compounds and were fully characterized by ^{1}H NMR spectral analysis. The ^{1}H NMR signals assigned by ^{1}H–^{1}H COSY, NOESY, and ^{1}H–^{1}H decoupling experiments to the polyene part of all-*E* and five di-*Z* isomers are compiled in Table 3. The geometry of the polyene chain was determined by isomerization shift values ($\Delta\delta = \Delta Z - \Delta E$) of ^{1}H NMR (Englert, 1995) and NOESY data (Table 3, Fig. 3). For example, in the 9*Z*,13*Z* isomer (**5**), ^{1}H signals from H-7, H-8, H-11, and H-12 were shifted strongly down-field with respect to those from the all-*E* isomer (**6**). However, the signals from H-10 and H-14 were shifted up-field relative to those of the all-*E* isomer (**6**). These isomerization shift patterns are characteristic of a 9*Z*,13*Z* geometry (Englert, 1995). Furthermore, the NOESY correlations H-19/H-7, H-19/H-10, H-20/H-11, and H-20/H-14 (Fig. 3) were also consistent with a 9*Z*,13*Z* geometry. In a similar manner as described above, the geometries of the other four di-*Z* isomers were also determined (Fig. 3).

Furthermore, seven known carotenoids were identified in extracts of chrysanthemum by UV–Vis, ^{1}H NMR, and FABMS data: (9*Z*)-violaxanthin (from peak 6), (8*S*)-lutein-5,8-epoxide (from peak 7), (8*R*)-lutein-5,8-epoxide (from peak 8), (9*Z*-8′*R*)-luteoxanthin (from peak 8), (all-*E*)-lutein (from peak 11), (9*Z*)-lutein (from peak 12), and (9′*Z*)-lutein (from peak 13) (Table 2). All of these carotenoids are classified as xanthophylls. (all-*E*)-Lutein, (9*Z*)-lutein, (9′*Z*)-lutein, (9*Z*)-violaxanthin, **6**, **8**, and **9** were previously reported in chrysanthemum by Tóth and Szabolcs (1981): we identified a further nine carotenoids in the present investigation. All of these carotenoids, except for (9*Z*)-violaxanthin, are β,ε-carotene (α-carotene) derivatives. Most species analyzed previously contain both α-carotene and β,β-carotene (β-carotene) derivatives. For example, among a Compositae plants reported so far, flowers of *Calendula officinalis* (Bakó et al., 2002), *Helianthus annus* (Tóth and Szabolcs, 1981; Deli et al., 1988), *Helianthus debilis* (Tóth and

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data for **1** in $CDCl_3$

Position	δ_C (mult.)	δ_H (mult., J_{Hz})	Position	δ_C (mult.)	δ_H (mult., J_{Hz})
1	38.7 (*s*)		1′	34.0 (*s*)	
2	45.2 (*t*)	α1.61 (*dd*)[b]	2′	44.6 (*t*)	α1.37 (*dd*, 13, 7)
		β1.83 (*dd*, 13.5, 6)			β1.85 (*dd*, 13, 5.5)
3	66.3 (*d*)	3.97 (*m*)	3′	65.9 (*d*)	4.25 (*m*)
4	43.9 (*t*)	1.92 (*m*)	4′	124.5 (*d*)	5.55 (*s*)
		1.92 (*m*)			
5	79.4 (*s*)		5′	138.0 (*s*)	
6	76.3 (*s*)		6′	55.0 (*d*)	2.40 (*d*, 10)
7	127.5 (*d*)	5.83 (*d*, 15.5)	7′	128.8 (*d*)	5.43(*dd*, 15.5, 10)
8	137.5 (*d*)	6.56 (*d*, 15.5)	8′	137.7 (*d*)	6.14 (*d*, 15.5)
9	134.1 (*s*)		9′	135.1 (*s*)	
10	132.9 (*d*)[a]	6.23 (*d*, 11.0)	10′	130.8 (*d*)	6.14 (*d*, 11)
11	124.6 (*d*)	6.62 (*dd*, 15.5, 15.5)	11′	124.9 (*d*)	6.62 (*dd*, 15.5, 11)
12	138.3 (*d*)	6.39 (*d*, 15.5)	12′	138.3 (*d*)	6.36 (*d* 15.5)
13	136.2 (*s*)		13′	136.6 (*s*)	
14	132.5 (*d*)[a]	6.25 (*m*)	14′	132.5 (*d*)	6.25 (*m*)
15	130.3 (*d*)	6.64 (*m*)	15′	130.0 (*d*)	6.64 (*m*)
16	26.9 (*q*)	1.03 (*s*)	16′	29.5 (*q*)	1.00 (*s*)
17	27.7 (*q*)	1.07 (*s*)	17′	24.3 (*q*)	0.85 (*s*)
18	27.7 (*q*)	1.36 (*s*)	18′	22.9 (*q*)	1.63 (*s*)
19	13.3 (*q*)	1.94 (*s*)	19′	13.1 (*q*)	1.92 (*s*)
20	12.8 (*q*)	1.97 (*s*)	20′	12.8 (*q*)	1.97 (*s*)

[a] Assignments may be interchanged.
[b] Signal overlapped.

Table 3
^{1}H (500 MHz) NMR spectral data relevant to the polyene part of (all-*E*)- and (di-*Z*)-lutein-5,6-epoxide in $CDCl_3$

Position	All-*E* (**6**)	9Z,9′Z (**7**)		9Z,13Z (**5**)		9Z,13′Z (**2**)		9′Z,13′Z (**4**)		13Z,9′Z (**3**)	
	δ	δ	Δδ[a]	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ
H-7	5.88	5.94	0.06	5.96	0.08	5.94	0.06	5.88		5.91	0.03
H-8	6.29	6.84	0.55	6.83	0.54	6.84	0.55	6.29		6.32	0.03
H-19	1.93	1.94		1.96	0.03	1.94		1.93		1.94	
H-10	6.20	6.08	−0.12	6.10	−0.10	6.08	−0.12	6.20		6.24	0.04
H-11	6.60	6.76	0.16	6.76	0.16	6.76	0.16	6.60		6.58	
H-12	6.38	6.30	−0.08	6.83	0.45	6.30	−0.08	6.38		6.70	0.32
H-20	1.97	1.97		1.99		1.96		1.97		1.99	
H-14	6.26	6.25		6.14	−0.12	6.19	−0.07	6.25		6.12	−0.14
H-15	6.66	6.63	−0.03	6.76	0.10	6.61	−0.05	6.56	−0.10	6.80	0.14
H-15′	6.66	6.63	−0.03	6.55	−0.11	6.78	0.12	6.80	0.14	6.60	−0.06
H-14′	6.26	6.25		6.23	−0.03	6.08	−0.18	6.12	−0.14	6.24	
H-20′	1.97	1.99		1.96		1.99		2.01	0.04	1.98	
H-12′	6.36	6.30	−0.06	6.36		6.88	0.52	6.82	0.46	6.30	−0.06
H-11′	6.60	6.74	0.14	6.60		6.56	−0.04	6.76	0.16	6.74	0.14
H-10′	6.14	6.03	−0.11	6.14		6.23	0.09	6.08	−0.06	6.03	−0.11
H-19′	1.91	1.91		1.91		1.92		1.93		1.91	
H-8′	6.14	6.67	0.53	6.14		6.16		6.67	0.53	6.67	0.53
H-7′	5.43	5.46	0.03	5.43		5.46	0.03	5.49	0.06	5.46	0.03

[a] Isomerization shift ($\Delta\delta = \delta E - \delta Z > 0.02$ ppm).

Szabolcs, 1981), and *T. erecta* (Khachik et al., 1999) have not only lutein and/or lutein derivatives as their major carotenoids but also several β-carotene derivatives such as antheraxanthin, zeaxanthin and violaxanthin. *T. erecta* flowers have the highest relative amount of α-carotene derivatives (i.e., approximately 92% of total carotenoids) among the plant species reported so far. Our investigation showed that the petals of chrysanthemum have a higher proportion of α-carotene derivatives than those of *T. erecta*. Moreover, we isolated various isomers, such as eight geometrical isomers of lutein-5,6-epoxide (i.e., the all-*E* form, two mono-*Z* forms, and five di-*Z* forms; Fig. 3), three geometrical isomers of lutein (the all-*E* form and two mono-*Z* forms), and two epimeric lutein-5,8-epoxides. The di-*Z* forms of cyclized carotenoids are particularly rare molecular structures and have been reported as natural products only in the petals of *Brassica napus* ((9*Z*,9′*Z*)-lutein), *C. officinalis* ((9*Z*,9′*Z*)-

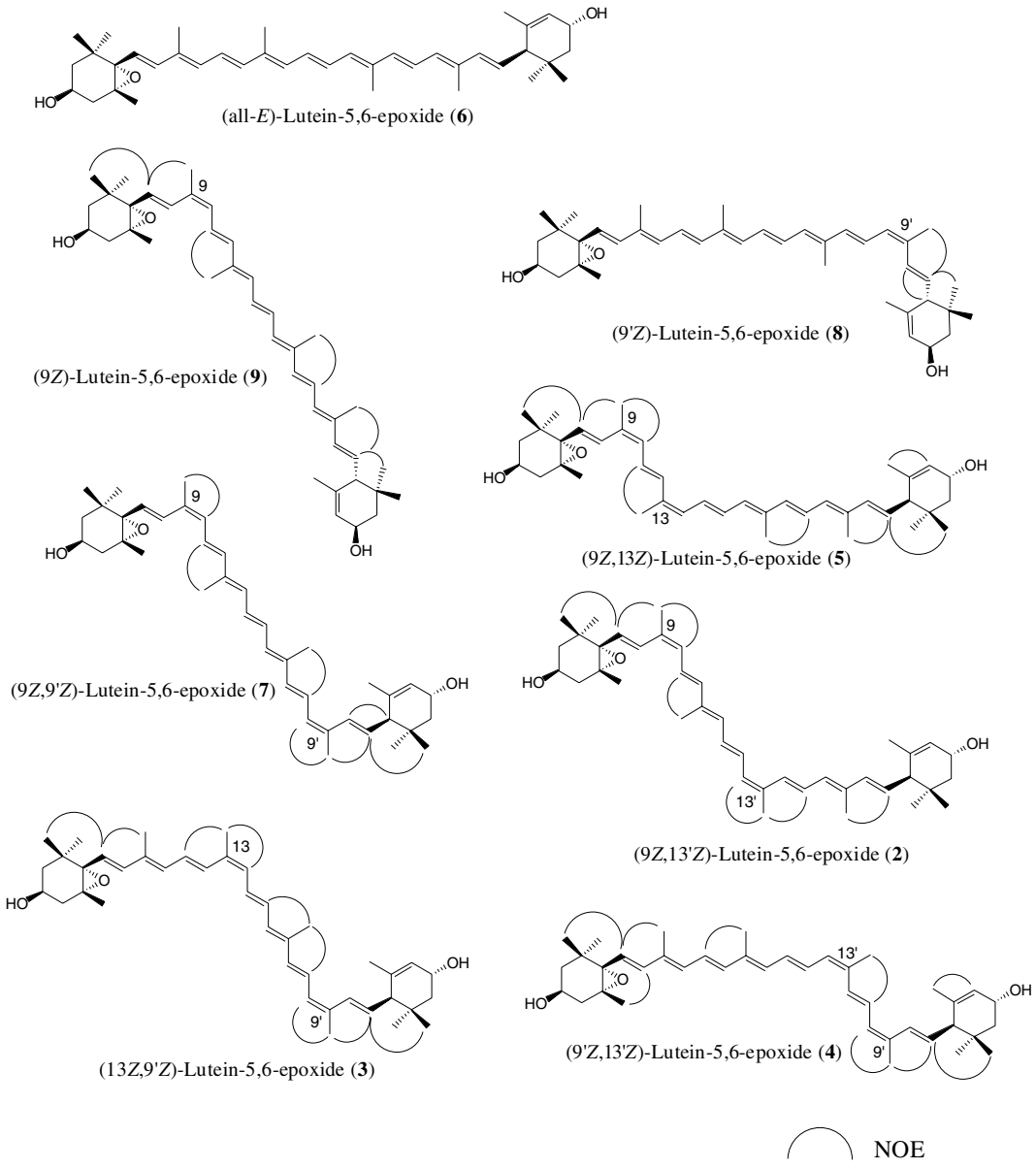

Fig. 3. Structures of eight geometrical isomers of lutein-5,6-epoxide identified in this study.

lutein and (13*Z*,13′*Z*)-lutein), *T. erecta* ((13*Z*,13′*Z*)-lutein), and *Viola tricolor* ((9*Z*,9′*Z*)-violaxanthin, (9*Z*,13*Z*)-violaxanthin, (9*Z*,13′*Z*)-violaxanthin, and (9*Z*,15*Z*)-violaxanthin; Molnár et al., 1986).

In conclusion, our results indicate that petals of chrysanthemum have a unique carotenoid composition in comparison with flowers of other species.

3. Experimental

3.1. Plant materials

Chrysanthemums (*D. grandiflorum*) were grown in greenhouses at the National Institute of Floricultural Science (Tsukuba, Ibaraki, Japan), and mature petals were harvested.

3.2. General experimental procedures

UV–Vis spectra were recorded in Et_2O with a Shimadzu UV-240 spectrophotometer or in the mobile phase of HPLC with a Jasco MD-915 photodiode array detector.

FABMS spectra were recorded with a Jeol SX 102 mass spectrometer with nitrobenzyl alcohol as the matrix. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured with a Varian Unity Inova 500 spectrometer in $CDCl_3$ with TMS as an internal standard. CD spectra were recorded in Et_2O at room temperature with

a Jasco J-500C spectropolarimeter. HPLC analysis was performed under the following conditions: column, YMC Carotenoid (250 × 20 mm i.d., 5 μm, YMC Co., Ltd.); solvent, MeOH–H_2O (96:4); flow rate, 10.0 mL min^{-1}; column temperature, 35 °C. HPLC preparation was performed under the following conditions: column, YMC Carotenoid (250 × 20 mm i.d., 5 μm, YMC Co., Ltd.); solvent, MeOH–H_2O (96:4); flow rate, 10.0 mL min^{-1}; column temperature, 35 °C; or column, ChemcoPak Si (300 × 7.8 mm i.d., 5 μm , Chemco Co., Ltd.); solvent, hexane–Me_2CO (7:3); flow rate, 2.0 mL min^{-1}.

3.3. Isolation of carotenoids

A Me_2CO extract of fresh petals (100 g) of chrysanthemum flowers was partitioned between Et_2O and aqueous NaCl. The organic layer was concentrated to dryness. The residue was saponified with 5% KOH–MeOH for 3 h at room temperature. Then the unsaponifiable matter was extracted with Et_2O and washed with water. The organic layer was dried over Na_2SO_4 and then concentrated to dryness. Compounds were purified and fractionated by HPLC on a Carotenoid column as described above. Peaks 2 and 4 were further purified by HPLC on a ChemcoPak Si column.

Sixteen carotenoids were identified in the extracts of chrysanthemum petals: (3*S*,5*S*,6*R*,3′*R*,6′*R*)-5,6-dihydro-5,6-dihydroxylutein (R_t 22.1 min); (9*Z*,13′*Z*)-lutein-5,6-epoxide (R_t 27.5 min); (13*Z*,9′*Z*)-lutein-5,6-epoxide (R_t 27.5 min); (9*Z*,13*Z*)-lutein-5,6-epoxide (R_t 30.7 min); (9′*Z*,13′*Z*)-lutein-5,6-epoxide (R_t 28.9 min); (all-*E*)-lutein-5,6-epoxide (R_t 40.6 min); (9*Z*,9′*Z*)-lutein-5,6-epoxide (R_t 47.2 min); (9*Z*)-violaxanthin (R_t 47.2 min); (8*S*)-lutein-5,8-epoxide (R_t 49.0 min); (8*R*)-lutein-5,8-epoxide (R_t 51.8 min); (9*Z*-8′*R*)-luteoxanthin (R_t 51.8 min); (9′*Z*)-lutein-5,6-epoxide (R_t 61.1 min); (9*Z*)-lutein-5,6-epoxide (R_t 64.6 min); (all-*E*)-lutein (R_t 78.0 min); (9*Z*)-lutein (R_t 98.4 min); and (9′*Z*)-lutein (R_t 125.0 min). They were identified by UV–Vis, ^1H NMR, and FABMS spectroscopy.

3.4. (3S,5S,6R,3′R,6′R)-5,6-Dihydro-5,6-dihydroxylutein (1)

Yield: ca. 2.0 mg from 100 g fresh petals; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Table 2; HRFABMS: Calc. for $C_{40}H_{56}O_4$ (M^+): 602.4335; Found: 602.4342; CDλ, ($\Delta\varepsilon$) in Et_2O: 320 (0), 290 (−0.5), 285 (0), 256 (4.6), 230 (2); UV–Vis: λ_{max} (Et_2O) nm: 265, 313, 327, 414, 438, 467; Key HMBC correlations: H-16 to C-1, C-2, C-6, H17 to C-1, C-2, C-6, H-18 to C-4, C-5, C-6; Key NOESY correlations: H-16/H-2α,H-16/H-17, H-18/H-3, H18/H17.

3.5. (9Z,13′Z)-Lutein-5,6-epoxide (2)

Yield: ca. 0.7 mg; ^1H NMR (500 MHz, $CDCl_3$): Table 3; HRFABMS: Calc. for $C_{40}H_{56}O_3$ (M^+):584.4230; Found: 584.4221; UV–Vis: λ_{max} (Et_2O) nm: 269, 314, 327, 406, 430, 457; % A_B/A_{II} = 32 [% A_B/A_{II} = percent of absorption intensity (A_B) at *Z*-peak in the near-UV region (327 nm) to absorption intensity (A_{II}) at main absorption maximum (Britton, 1995; Khachik et al., 1999)].

3.6. (13Z,9′Z)-Lutein-5,6-epoxide (3)

Yield: ca. 0.7 mg; ^1H NMR (500 MHz, $CDCl_3$): Table 3; HRFABMS: Calc. for $C_{40}H_{56}O_3$ (M^+):584.4230; Found: 584.4219; UV–Vis: λ_{max} (Et_2O) nm: 270, 314, 327, 405, 4298, 454; % A_B/A_{II} = 40.

3.7. (9′Z,13′Z)-Lutein-5,6-epoxide (4)

Yield: ca. 0.9 mg; ^1H NMR (500 MHz, $CDCl_3$): Table 3 ; HRFABMS: Calc. for $C_{40}H_{56}O_3$ (M^+):584.4230; Found: 584.4235; UV–Vis: λ_{max} (Et_2O) nm: 267, (315), (327), 415, 438, 469; % A_B/A_{II} = 9.

3.8. (9Z,13Z)-Lutein-5,6-epoxide (5)

Yield: ca. 0.8 mg; ^1H NMR (500 MHz $CDCl_3$): Table 3; HRFABMS: calc. for $C_{40}H_{56}O_3$ (M^+):584.4230; Found: 584.4224; UV–Vis: λ_{max} (Et_2O) nm: 267, (315), (327), 415, 438, 469; % A_B/A_{II} = 9.

3.9. (9Z,9′Z)-Lutein-5,6-epoxide (7)

Yield: ca. 1.0 mg; ^1H NMR (500 MHz, $CDCl_3$): Table 3; HRFABMS: Calc. for $C_{40}H_{56}O_3$ (M^+):584.4230; Found: 584.4239; UV–Vis: λ_{max} (Et_2O) nm: 266, (311), (326), 407, 430, 458; % A_B/A_{II} = 2.

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