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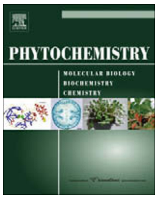

Sterically hindered carotenoids with 3Z, 5Z configuration from the seeds of oriental bitter sweet, *Celastrus orbiculatus*

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ABSTRACT

Sterically hindered *cis*-carotenoids **1** and **2** were isolated from seeds of the oriental bitter sweet, *Celastrus orbiculatus*. Their structures were determined to be (3′*Z*, 5′*Z*)-celaxanthin and (3′*Z*, 5′*Z*)-torulene, respectively, on the basis of spectroscopic data and iodine-catalyzed stereomutation. This is the first report on carotenoids with a 3*Z*, 5*Z* configuration.

1. Introduction

More than 700 carotenoids of extensive structural variety (Britton et al., 2004) have been isolated in nature. This previously prompted our research group to search for new carotenoids from various natural sources (Maoka, 2009) and several interesting structural carotenoids were also isolated from seeds of the plant, *Pittosporum tobira* (Pittosporaceae) (Maoka et al., 2007, 2008).

The oriental bitter sweet, *Celastrus orbiculatus* Thumb. (Celastraceae), is a deciduous vine that grows in Japan. It has been used in Chinese folk medicine as a treatment for rheumatoid arthritis and bacterial infections (Zhu et al., 2008). Several new sesquiterpenes, having cytotoxic activity, were isolated from its seeds (Zhu et al., 2008).

The seeds are encased in yellow pods that break open during autumn, when the seeds are exposed to sunlight and change color from yellow to red. In the course of our recent carotenoid study in plants (Maoka et al., 2007, 2008), we isolated two new carotenoids, **1** and **2**, having a sterically hindered *cis* polyene chain (Fig. 1), from the seeds of *C. orbiculatus*. This paper reports the isolation and structural elucidation of these two new carotenoids.

2. Results and discussion

The seeds of *C. orbiculatus* (1 kg) were extracted with MeOH. The MeOH extract was saponified with 5% KOH–MeOH at room temperature. The non-saponifiable matter was subjected to silica gel column chromatography followed by additional chromatography using MgO–Celite (1:1), successively, to obtain **1** (10 mg) and **2** (1.8 mg).

The new carotenoid **1** was isolated as red needles, and its visible light absorption spectrum showed absorption maxima at 465 and 490 (shoulder) nm. The molecular formula was determined to be $C_{40}H_{54}O$ by HREIMS. The presence of a secondary hydroxyl group was consistent with the formation of a monoacetate of **1** and with analysis of the ^{1}H NMR (δ 4.00) and ^{13}C NMR (δ 65.1) spectroscopic data (Englert, 1995). The ^{1}H NMR spectrum displayed resonances for 30 methyl protons, four methylene protons, one oxymethine proton, and 18 olefinic protons. The ^{13}C NMR spectrum, including a DEPT experiment, showed forty carbon signals: ten CH_3, two CH_2, one CH, eighteen alkene CH, one sp^3 quaternary carbon, and eight sp quaternary carbon resonances (Table 1). The assignments of the ^{1}H and ^{13}C NMR spectra for **1** were accomplished by COSY, ROESY, HSQC, and HMBC analyses. These ^{1}H and ^{13}C NMR spectroscopic data were almost the same as those of (all *E*)-celaxanthin, except for the C-1′ to C-8′ positions (Table 1).

The IR absorption at 770 cm^{-1} indicated the presence of a *cis* alkene group in **1** (Bernhard and Grosjean, 1995). Iodine-catalyzed stereomutation of **1** gave mainly (all *E*)-celaxanthin with a bathochromic shift of 15 nm in the visible absorption spectrum. These properties indicated that **1** was a sterically hindered *cis* isomer (Clough and Pattenden, 1979, 1983) of celaxanthin.

The ^{1}H NMR signals of H-2′, H-3′, H-4′, H-6′, H-7′, H-8′, and H-18′ of **1** were very similar to those of H-6, H-7, H-8, H-10, H-11, H-12, and H-19 of prolycopene (Englert 1979, 1995; Hengartner et al., 1992; Pattenden and Robson 1987, 2006), having a 7*Z*, 9*Z*

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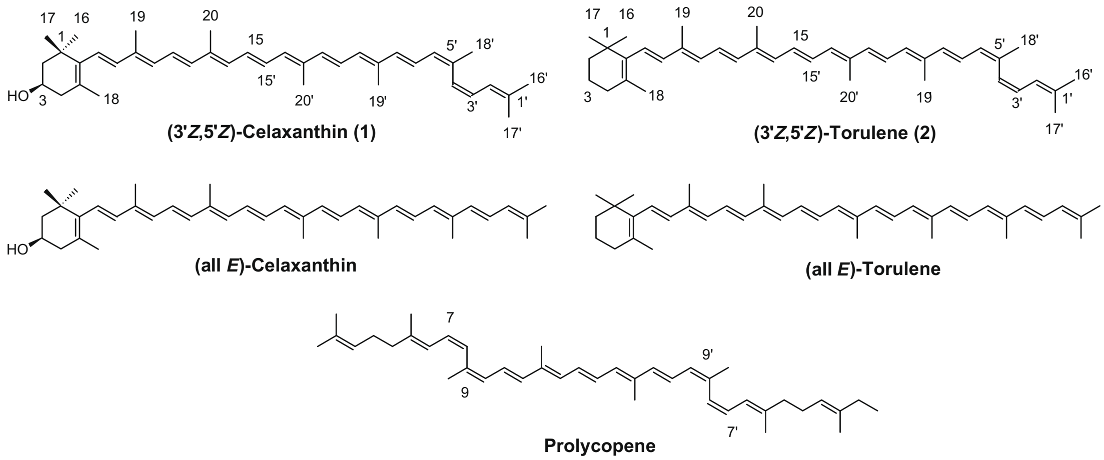

Fig. 1. Structure of carotenoids.

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of (3′Z,5′Z)-celaxanthin (**1**) and (all *E*)-celaxanthin.

Position	(3′Z,5′Z)-celaxanthin			(all E)-celaxanthin		
	δ ^{13}C	δ ^1H	mult. (*J*. Hz)	δ ^{13}C	δ ^1H	mult. (*J*. Hz)
1	37.1			37.1		
2	48.5	1.48	*dd* (12, 12)	48.4	1.48	*dd* (12, 12)
		1.78	*ddd* (12, 5, 2)		1.78	*ddd* (12, 5, 2)
3	65.1	4.00	*m*	65.1	4.00	*m*
4	42.6	2.04	*dd* (16, 10)	42.5	2.04	*dd* (16, 10)
		2.39	*dd* (16, 6, 2)		2.39	*dd* (16, 6, 2)
5	126.2			126.2		
6	137.6			137.7		
7	125.6	6.10	*d* (16)	125.6	6.10	*d* (16)
8	138.6	6.15	*d* (16)	138.6	6.15	*d* (16)
9	135.7			135.7		
10	131.4	6.16	*d* (11.5)	131.4	6.16	*d* (11.5)
11	124.9	6.63	*dd* (15, 11.5)	124.9	6.63	*dd* (15, 11.5)
12	137.5	6.36	*d* (15)	137.5	6.36	*d* (15)
13	136.5			136.5		
14	132.6	6.26	*d* (12)	132.6	6.26	*d* (12)
15	130.2	6.63	*m*	130.2	6.63	*m*
16	28.8	1.08	*s*	28.7	1.08	*s*
17	30.3	1.08	*s*	30.2	1.08	*s*
18	21.6	1.74	*s*	21.6	1.74	*s*
19	12.8	1.97	*s*	12.8	1.97	*s*
20	12.8	1.97	*s*	12.8	1.97	*s*
1′	136.6			136.0		
2′	123.0	6.11	*d* (11.5)	131.3	5.94	*d* (11.5)
3′	126.2	6.28	*dd* (11.5, 11.5)	125.6	6.47	*dd* (15, 11.5)
4′	126.5	6.01	*d* (11.5)	135.0	6.23	*d* (15)
5′	135.5			137.4		
6′	129.8	6.03	*d* (11.5)	131.3	6.18	*d* (11.5)
7′	126.2	6.49	*dd* (15, 11.5)	126.0	6.62	*dd* (15, 11.5)
8′	136.1	6.27	*d* (15)	137.6	6.38	*d* (15)
9′	136.4			136.3		
10′	130.1	6.20	*d* (11.5)	130.1	6.18	*d* (11.5)
11′	131.4	6.63	*dd* (15, 11.5)	125.2	6.65	*dd* (15, 11.5)
12′	125.2	6.36	*d* (15)	137.9	6.36	*d* (15)
13′	136.4			136.5		
14′	132.7	6.26	*d* (12)	132.7	6.26	*d* (12)
15′	130.2	6.63	*m*	130.1	6.63	*m*
16′	26.5	1.80	*s*	26.3	1.82	*s*
17′	18.1	1.80	*s*	18.6	1.82	*s*
18′	24.8	2.00	*s*	12.9	1.94	*s*
19′	12.7	1.90	*s*	12.7	1.97	*s*
20′	12.7	1.97	*s*	12.7	1.97	*s*

s: singlet, *d*: doublet, *m*: multiplet.

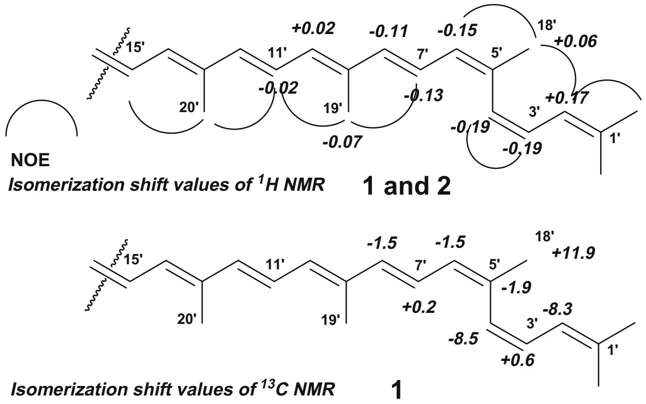

Fig. 2. ROESY correlations and isomerization shift ($\Delta\delta = \delta_Z - \delta_E$) values of ^1H NMR of **1** and **2** (upper) and isomerization shift values of ^{13}C NMR of **1** (lower).

(7′*Z*, 9′*Z*) configuration, respectively. The ^{13}C NMR signals of C-2′ to C-8′ and C-18′ of **1** were also similar to those of C-6′ to C-12′ and C-19 of prolycopene (Englert et al., 1979; Hengartner et al., 1992), respectively. These NMR data also suggested that **1** was a 3′*Z*, 5′*Z* isomer of celaxanthin.

The position of the *cis* double bonds in **1** was determined by isomerization shift ($\Delta\delta = \delta_Z - \delta_E$) values of ^1H and ^{13}C NMR signals (Englert, 1979, 1995; Englert et al., 1979; Hengartner et al., 1992; Pattenden and Robson, 1987, 2006) and NOE correlations obtained from the ROESY spectrum, as shown in Fig. 2. The downfield shift of ^1H NMR signals at H-2′ and H-18′, and high field shift at H-3′, H-4′, H-6′, H-7, and H-8′ in **1** from the corresponding signals of (all *E*)-celaxanthin were observed (Fig. 2). These isomerization shift values were in agreement with the 3′*Z*, 5′*Z* configuration. This was confirmed by NOE correlations obtained from the ROESY spectrum. The NOE correlations between H-3′ and H-4′, between H-18′ and H-2′, between H-18′ and H-6′, and between H-19′ and H-7′ clearly indicated a 3′*Z*, 5′*Z* configuration for **1** (Fig. 2). The ^{13}C NMR signals of **1** also showed a marked down field shift at C-18′ (11.9 ppm) and a high field shift at C-2′, C-4′, C-6′, and C-8′ from the corresponding resonances of (all *E*)-celaxanthin. These isomerization shift values were also compatible with the 3′*Z*, 5′*Z* configuration (Fig. 2). Therefore, the structure of **1** was determined to be (3′*Z*, 5′*Z*)-celaxanthin.

Compound **2** had the same absorption spectrum as that of **1**, and its molecular formula was determined to be $C_{40}H_{54}$ by HREIMS. As for **1**, iodine-catalyzed stereomutation of **2** gave mainly (all *E*)-torulene with a bathochromic shift of 15 nm in the visible absorption spectrum. The ^1H NMR spectrum of **2** showed the presence of half (H-2 to H-20) of a β-carotene moiety and another half of (3′*Z*, 5′*Z*)-celaxanthin (H-2′ to H-20′) described above. These