Capsanthone 3,6-Epoxide, a New Carotenoid from the Fruits of the Red Paprika *Capsicum annuum* L.

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The structure of a new carotenoid, isolated from the fruits of the red tomato-shaped paprika *Capsicum annuum* L., was elucidated to be (3*S*,5*R*,6*S*,5′*R*)-3,6-epoxy-5,6-dihydro-5-hydroxy-β,κ-carotene-3′,6′-dione by spectroscopic analyses, including fast atom bombardment collision-induced dissociation–mass spectrometry/mass spectrometry (FAB CID–MS/MS) and was designated capsanthone 3,6-epoxide. Capsanthone 3,6-epoxide is assumed to be an oxidative metabolite of capsanthin 3,6-epoxide in paprika.

Keywords: *Capsicum annuum; carotenoid; capsanthone 3,6-epoxide; FAB CID–MS/MS*

INTRODUCTION

Ripe fruits of red paprika (red pepper) are used widely as vegetables and also as food colorants because they are a good source of carotenoid pigments. The red carotenoids in pepper (*Capsicum annuum* L.) are mainly capsanthin, capsorubin, and capsanthin 5,6-epoxide, possessing a 3-hydroxy κ-end group (*1–3*). At the same time, the fruits are also rich in yellow xanthophylls such as β-cryptoxanthin, zeaxanthin, violaxanthin, and antheraxanthin, as well as β-carotene (*4, 5*). Furthermore, many other carotenoids with interesting structures, especially those with the 3,5,6-trihydroxy-5,6-dihydro β-end group (karpoxanthin) (*6*), 3,4-didehydro-6-hydroxy γ-end group (nigroxanthin) (*7*), and 5-hydroxy-5,6-dihydro-3,6-epoxy-β-(oxabicyclo) end groups (cycloviolaxanthin, cucurbitaxanthins, and capsanthin 3,6-epoxide) (*8–12*) have been isolated. Deli et al. also isolated capsanthone possessing a 3-oxo κ-end group from paprika and determined its absolute configuration to be 3*R*, 5′*R* (*13*).

In the course of our studies on paprika carotenoids, eleven apocarotenoids, including five new compounds, were previously isolated from the fruits of the red tomato-shaped paprika *C. annuum* L. as minor components, along with eighteen known C_{40} carotenoids (*14*). During the isolation of these apocarotenoids, another new C_{40} carotenoid (**1**) was isolated as a minor component. This paper reports the isolation and the structural elucidation of the new carotenoid (**1**) by spectroscopic analysis, including the FAB CID–MS/MS method (*15*). Furthermore, possible biosynthetic ways for the formation of **1** were discussed.

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MATERIALS AND METHODS

Apparatus. The UV–Vis spectra were recorded with a Shimadzu UV-240 spectrophotometer in Et_2O. The EI-MS and positive ion FAB-MS spectra were recorded using a JEOL JMS-HX/HX 110A mass spectrometer. CID–MS/MS was performed using a JEOL JMS-HX/HX 110A four-sector tandem mass spectrometer equipped with a FAB gun operated at 6kV. A few μg of sample dissolved in benzene was placed on a stainless steel probe tip, and 1–2 μL of *m*-nitrobenzyl alcohol was added as a matrix. The sample was bombarded with xenon atoms, and ions produced were accelerated through 10keV. The radical cation $M^{+\cdot}$ selected as a precursor by MS1 was subjected to collision with argon gas in the collision cell, floated at 3 kV potential, between MS1 and MS2. The amount of argon gas was adjusted to attenuate the intensity of the precursor ion by 30%. The CID spectra were obtained by linked scanning at constant B/E on MS2.

The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured with a Varian UNITY *INOVA* 500 spectrometer in $CDCl_3$ with TMS as an internal standard. DQF–COSY, NOESY (mixing time 1.3 s), gHSQC ($^1J_{CH}$ = 142 Hz), and gHMBC ($^nJ_{CH}$ optimized for 8 Hz) spectra were acquired using the standard Varian pulse programs; and the software used to obtain 2-D spectra was from Varian, version 6.1A. The CD spectra were recorded in Et_2O at room temperature with a JASCO J-500 spectropolarimeter.

Semipreparative HPLC was performed on a Shimadzu LC-6AD instrument with a Shimadzu SPD-6AV spectrophotometer set at 380 nm. The column used was a Lichrospher 100 RP-18 (Cica Merck, 20 mm × 250 mm, 10 μm) using CH_2Cl_2/CH_3CN (5:95) as the mobile phase and a flow rate of 5 mL/min.

Plant Material. The matured fruits of the red tomato-shaped paprika (*Capsicum annuum* L.) were collected from pepper plants in September. The pepper plants were grown in a greenhouse at a farm in Hitachinaka, Ibaraki Prefecture, Japan.

Extraction and Isolation of Carotenoids. The MeOH extract of the fresh fruits (800 g) of *C. annuum* L. was partitioned between *n*-hexane/Et_2O (1:1) 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 unsaponifiable matter was extracted with *n*-hexane/Et_2O (1:1) and washed with water. The organic layer was dried over Na_2SO_4 and then concentrated to dryness. The residue was subjected to silica-gel column chromatography

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Table 1. ^{13}C (125 MHz) and ^{1}H NMR (500 MHz) Data for Capsanthone 3,6-epoxide (1) in $CDCl_3$

	δ_C	δ_H mult. J (Hz)		δ_C	δ_H mult. J (Hz)
1	44.0	-	1′	41.1	-
2	48.5	1.62 d (11.5), 1.85 ddd (11.5, 6, 2)	2′	52.4	2.24,*[a] 2.28*
3	75.4	4.39 t like (6)	3′	216.4	-
4	47.7	1.68 d (12), 2.06 ddd (12, 6, 2)	4′	48.2	2.08 d (18.5), 3.10 d (18.5)
5	82.5	-	5′	55.8	
6	91.7	-	6′	201.2	-
7	123.2	5.76 d (16)	7′	119.7	6.49 d (15)
8	134.8	6.38 d (16)	8′	148.3	7.41 d (15)
9	135.7	-	9′	133.6	-
10	131.6	6.20 d (11)	10′	141.8	~6.60
11	125.4	6.72 dd (15, 11)	11′	123.9	~6.59
12	137.6	6.37 d (15)	12′	142.6	~6.54
13	135.7	-	13′	137.6	-
14	132.4	6.27 d (11.5)	14′	135.7	6.36 d (11.5)
15	129.7	6.65 m	15′	131.5	6.65 m
16	32.2	1.44 s	16′	25.3	1.01 s
17	25.7	0.89 s	17′	24.8	1.24 s
18	31.6	1.21 s	18′	19.8	1.39 s
19	12.9	1.96 s	19′	12.9	1.96 s
20	12.9	1.98 s	20′	12.9	1.97 s

[a] * indicates an AB spin system.

using an increasing percentage of Me_2CO in *n*-hexane. A new carotenoid (**1**) was eluted with Me_2CO/*n*-hexane (2:8) from the silica gel column with a series of apocarotenoids previously reported (*14*) and was further purified by HPLC on a C_{18} reversed-phase column with CH_2Cl_2/CH_3CN (5:95) as the solvent (*14*).

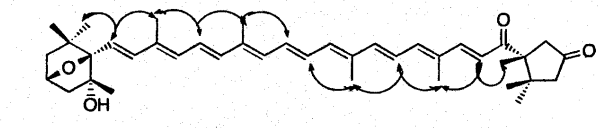
Capsanthone 3,6-epoxide (1). Yield 1 mg (0.4% of the total carotenoid); retention time on HPLC (Rt) 15 min; UV–Vis λ_{max} (Et_2O) 470 nm; high-resolution EI-MS *m/z* [$M^{+\cdot}$] 598.4028 ($C_{40}H_{54}O_4$ calcd 598.4022); CD (Et_2O) λ ($\Delta\epsilon$) 243 (−0.5), 278 (+2), 325 (−1) nm; ^{1}H NMR and ^{13}C NMR (Table 1).

The following additional carotenoids were isolated from the matured fruits of the red tomato-shaped paprika (*C. annuum* L.): β-carotene (10 mg, 4% of total carotenoid), β-cryptoxanthin (8 mg, 3%), α-cryptoxanthin (1 mg, 0.4%), cryptocapsin (2 mg 0.8%), cycloviolaxanthin (4 mg, 1.6%), cucurbitaxanthin A (20 mg, 8%), cucurbitachrome (10 mg, 4%), zeaxanthin (50 mg, 18%), capsanthin 3,6-epoxide (8 mg, 3%), capsanthone (2 mg, 0.8%), capsanthin (100 mg, including geometrical isomers, 38%), capsorubin (10 mg, including geometrical isomers, 4%), antheraxanthin (5 mg, 2%), mutatoxanthin (2 mg, 0.8%), violaxanthin (3 mg, 1.2%), luteoxanthin (2 mg, 0.8%), auroxanthin (2 mg, 0.8%), neoxanthin (1 mg, 0.1%), apo-8′-zeaxanthinal (1 mg, 0,4%), apo-10′-zeaxanthinal (1 mg, 0.4%), apo-12′-zeaxanthinal (1 mg, 0.4%), apo-14′-zeaxanthinal (0.5 mg, 0.2%), apo-15-zeaxanthinal (0.1 mg. 0.04%), apo-13-zeaxanthinone (2 mg, 0.8%), apo-11-zeaxanthinal (0.5 mg, 0.2%), apo-9-zeaxanthinone (0.2 mg, 0.08%), apo-12′-capsorubinal (0.5 mg, 0.2%), apo-8′-capsorubinal (0.2 mg, 0.08%), and 9,9′-diapo-10,9′-*retro*-carotene-9,9′-dione (2 mg, 0.8%) (*14*). They were identified by UV–Vis, EI-MS, ^{1}H NMR, and CD spectral data.

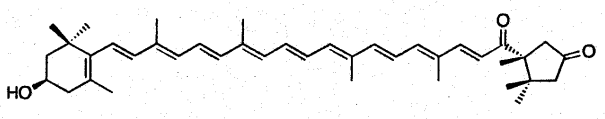
RESULTS AND DISCUSSION

The MeOH extract of the matured fruits of the red tomato-shaped paprika, *C. annuum* (800 g) was saponified with 5% KOH/MeOH, and unsaponifiable matter was chromatographed on silica gel using an increasing percentage of Me_2CO in *n*-hexane. Successive purification by semipreparative HPLC on a C_{18} reversed-phase column of the fraction eluted with Me_2CO/*n*-hexane (2:8) from a silica gel column afforded a new carotenoid (**1**).

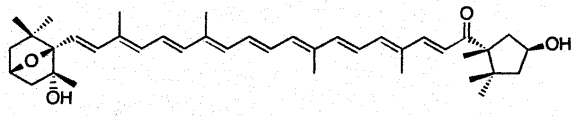
Compound **1** showed an absorption maximum at 470 nm without fine structure, which resembled that of capsanthin 3,6-epoxide. A high-resolution EI-MS established the molecular formula $C_{40}H_{54}O_4$. Of the four



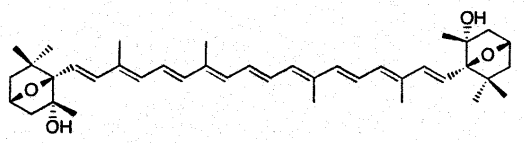
Capsanthone 3,6-epoxide (1)



Capsanthone



Capsanthin 3,6-epoxide



Cycloviolaxanthin

Figure 1. Structure and key NOESY correlations of capsanthone 3,6-epoxide (**1**) and structures of related carotenoids.

oxygen functions, two of them were ascribed to carbonyl groups (δ_C 201.2 and 216.4) and one to a tertiary hydroxy group (δ_C 82.5) by ^{13}C NMR data. From consideration of the high-resolution EI-MS and ^{13}C NMR (δ_C 75.4 and 91.7) data, the remaining oxygen was attributed to an epoxide group. The partial structure of the 5-hydroxy-5,6-dihydro-3,6-epoxy β-end group, 3-oxo κ-end group, and the polyene chain in **1** were characterized by ^{1}H and ^{13}C NMR including DQF–COSY, NOESY, HSQC, and HMBC experiments (*9, 13, 16*). From the spectral data described above, the structure of **1** was deduced to be 3,6-epoxy-5,6-dihydro-5-hydroxy-β,κ-carotene-3′,6′-dione and was designated capsanthone 3,6-epoxide. The all-*E* geometry of the polyene chain and relative stereochemistry of each end group were confirmed by NOESY data as shown in Figure 1. The 3*S*,5*R*,6*S*,5′*R* configuration for **1** was proposed from CD spectral data by comparison with those of (3*S*,5*R*,6*S*,3*S*′,5*R*′,6*S*′)-cycloviolaxanthin and

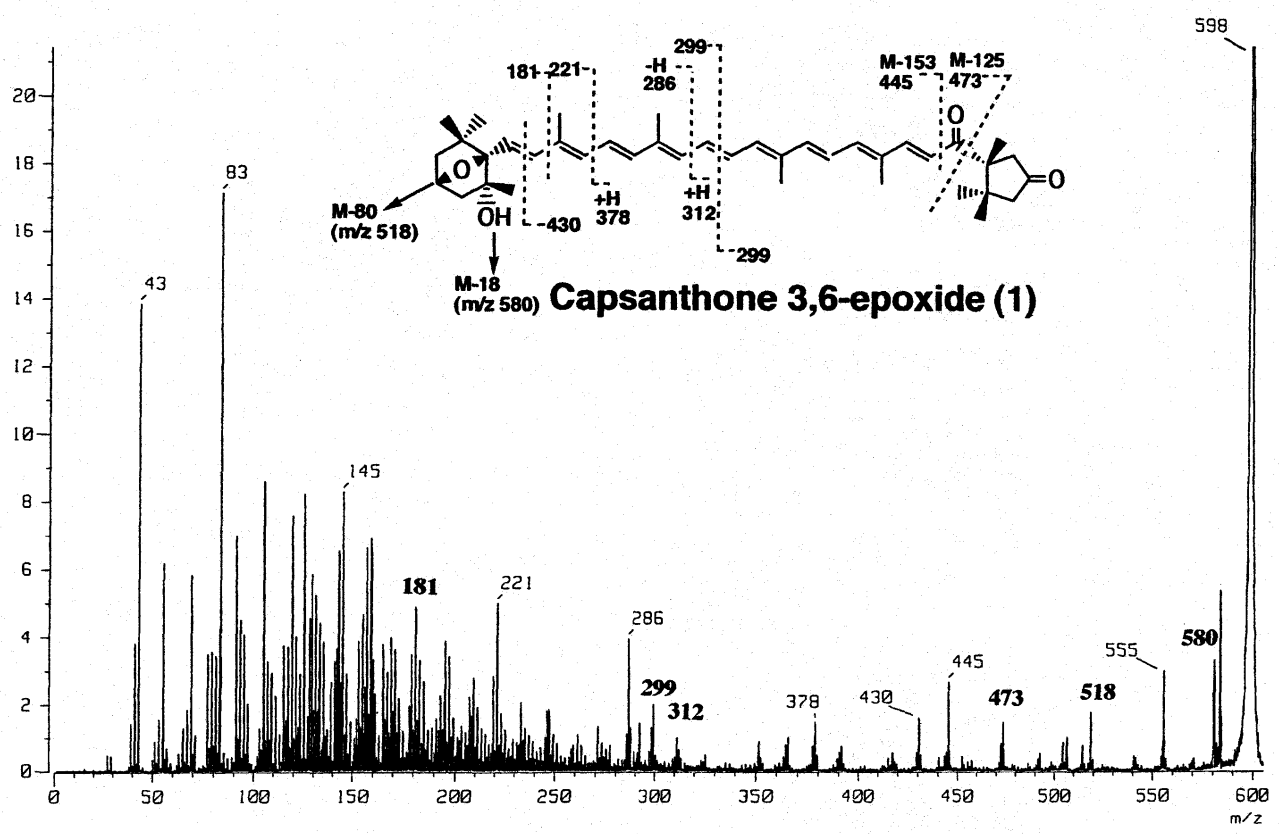


Figure 2. Positive ion FAB CID–MS/MS of capsanthone 3,6-epoxide (1).

(3*S*,5*R*,6*S*,3′*S*,5′*R*)-capsanthin 3,6-epoxide (*9*,*20*). Compound 1 showed almost the same CD spectrum as that of capsanthin 3,6-epoxide.

The positive ion FAB CID–MS/MS spectrum of the molecular ion ($M^{+\bullet}$) at *m/z* 598 of capsanthone 3,6-epoxide (1) is shown in Figure 2. Capsanthone 3,6-epoxide (1) showed product ions at *m/z* 580 $[M-18]^{+\bullet}$ and *m/z* 518 $[M-80]^{+\bullet}$, a characteristic fragment ion observed in epoxy carotenoids (*14*, *16*, *17*). Furthermore, a series of product ions resulting from cleavage of C–C bonds in polyene chain from the epoxy end group (i.e., *m/z* 181, 221, 286, 299, 445, and 473) were observed. The product ions at *m/z* 473 $[M-125]^{+\bullet}$ (attributed to cleavage between C-6′ and C-5′) and *m/z* 445 $[M-153]^{+\bullet}$ (attributed to cleavage between C-7′ and C-6′) indicated the presence of 3-oxo κ-end group in 1 (*19*). Moreover, ions produced by cleavage of C–C bonds in the polyene chain from the 3-oxo κ-end group site, such as *m/z* 299, 312, 378, and 430 (cleavage of double bond), were also observed. Among them, product ions at *m/z* 312 (attributed to cleavage between C-14 and C-15 and transfer of hydrogen to C-15 site), 378 (attributed to cleavage between C-9 and C-10 and transfer of hydrogen to C-10 site), and 430 (attributed to cleavage double bond between C-7 and C-8) were diagnostic ions of the 3,6-epoxy carotenoids (*14*). Therefore, the nonstereochemical structure of 1 could also be characterized from FAB CID–MS/MS data.

Capsanthone 3,6-epoxide (1) may be formed from capsanthin 3,6-epoxide by oxidation of a hydroxy group at C-3′ or from capsanthone by epoxidation at 5,6 position followed by rearrangement of the formation of a 3,6-epoxy end group (*21*, *22*). However, capsanthone 5,6-epoxide, a hypothetical possible intermediate from capsanthone to capsanthone 3,6-epoxide, has not been isolated in paprika. Therefore, 1 is assumed to be an oxidative metabolite of capsanthin 3,6-epoxide in paprika.

In conclusion, a new carotenoid, capsanthone 3,6-epoxide (1), possessing a 3-oxo κ-end group was isolated from the fresh fruits of the red tomato-shaped paprika *C. annuum* along with capsanthone.

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