# 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 *μ*g of sample dissolved in benzene was placed on a stainless steel probe tip, and 1−2 *μ*L 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 $M^{+\bullet}$ 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 $^1$H 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 *μ*m) 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 $CDCl_3$**

| | $\delta_C$ | $\delta_H$ mult. J (Hz) | | $\delta_C$ | $\delta_H$ 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 $\lambda_{max}$ ($Et_2O$) 470 nm; high-resolution EI-MS *m*/*z* [$M^{+\bullet}$] 598.4028 ($C_{40}H_{54}O_4$ calcd 598.4022); CD ($Et_2O$) $\lambda$ ($\Delta\epsilon$) 243 (−0.5), 278 (+2), 325 (−1) nm; $^1H$ 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, $^1H$ 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

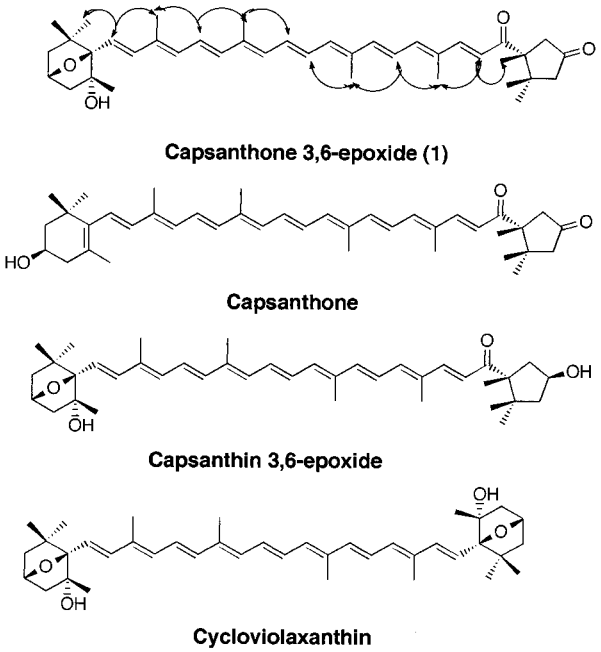


**Figure 1.** Structure and key NOESY correlations of capsanthone 3,6-epoxide (**1**) and structures of related carotenoids.

oxygen functions, two of them were ascribed to carbonyl groups ($\delta_C$ 201.2 and 216.4) and one to a tertiary hydroxy group ($\delta_C$ 82.5) by $^{13}C$ NMR data. From consideration of the high-resolution EI-MS and $^{13}C$ NMR ($\delta_C$ 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 $^1H$ 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

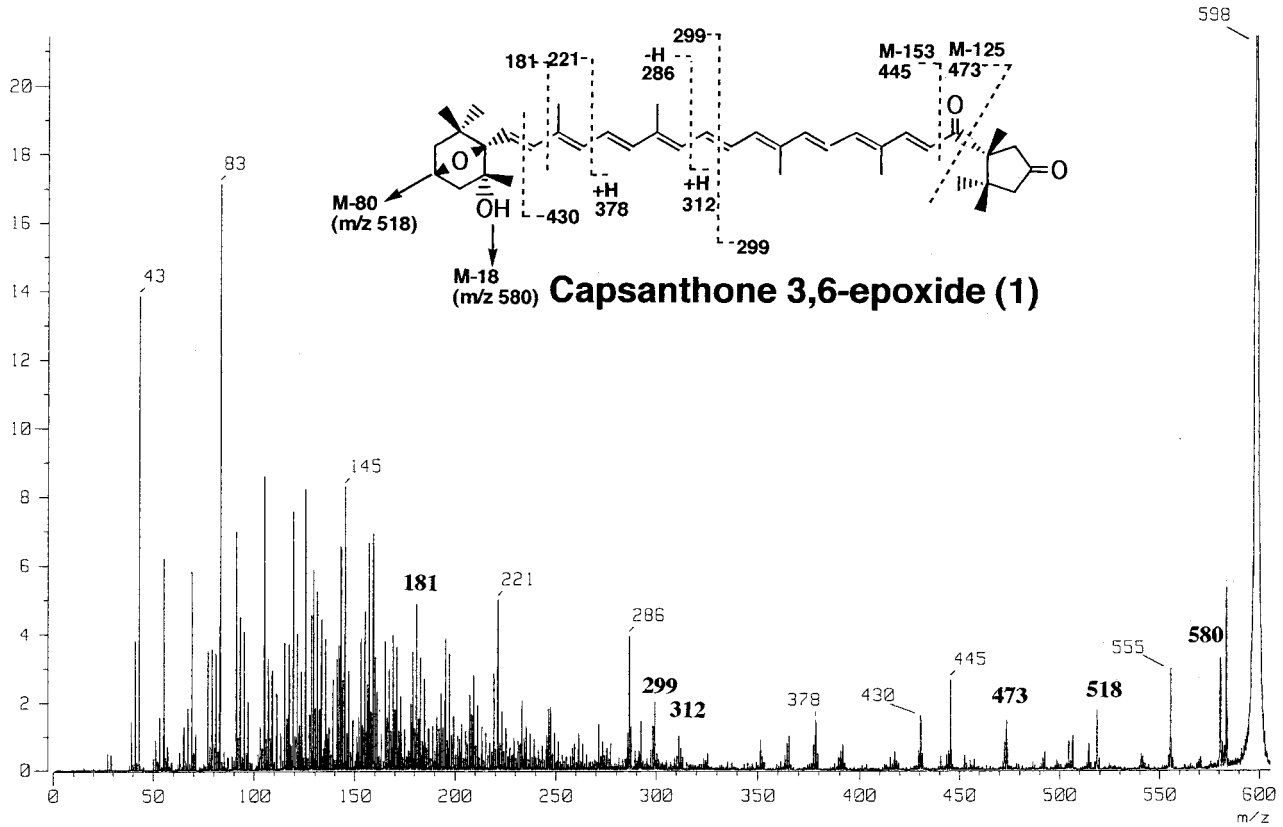


**Figure 2.** Positive ion FAB CID−MS/MS of capsanthone 3,6-epoxide (**1**).

(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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