Structure of New Carotenoids with the 6-Oxo-κ End Group from the Fruits of Paprika, *Capsicum annuum*

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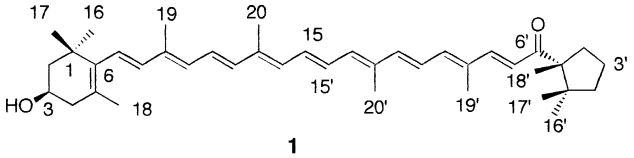
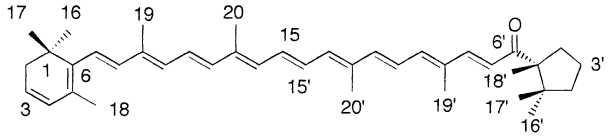
New carotenoids **1** and **2** were isolated as minor components from the ripe fruits of paprika (*Capsicum annuum*). The structures of **1** and **2** were determined to be (3*R*,5′*R*)-3-hydroxy-β,κ-caroten-6′-one and (5′*R*)-3,4-didehydro-β,κ-caroten-6′-one, respectively, from UV−vis, NMR, CD, HRFABMS, and FABMS/MS spectra.

Ripe fruits of paprika (red pepper) are used widely as vegetables and food colorants and are a good source of carotenoid pigments. The red carotenoids in paprika, *Capsicum annuum* L. (Solanaceae), are mainly capsanthin, capsorubin, and cryptocapsin having a 3-hydroxy-6-oxo-κ end group and capsanthone having a 3,6-dioxo-κ end group.[1] Many other carotenoids, especially those with the 3,5,6-trihydroxy-5,6-dihydro-β end group (karpoxanthin[2]), 3,4-didehydro-6-hydroxy-γ end group (nigroxanthin[3] and prenigroxanthin[4]), and 5-hydroxy-5,6-dihydro-3,6-epoxy-β end group (capsanthin 3,6-epoxide,[5] cycloviolaxanthin,[6] cucurbitaxanthins,[6] and capsanthone 3,6-epoxide[7]), have been isolated from paprika.

In the course of studies on the carotenoids of paprika,[7,8] two new carotenoids, **1** and **2**, which possess a 6-oxo-κ end group were isolated as minor components. This paper reports the isolation and structural elucidation of these two new carotenoids.



1



2

The MeOH extract of ripe fruits of paprika (4 kg) was saponified with 5% KOH−MeOH, and unsaponifiable matter was chromotographed on silica gel using an increasing percentage of acetone (Me_2CO) in hexane. The fraction eluted with Me_2CO−hexane (1:9) was subjected to HPLC on ODS with $CHCl_3$−MeCN (1:9) and then on silica gel with Me_2CO−hexane (2:8) to yield **1** (2 mg) and **2** (0.5 mg).

Compound **1** showed an absorption maximum at 465 nm without a fine structure, resembling capsanthin.[9] The molecular formula of **1** was determined to be $C_{40}H_{56}O_2$ by HRFABMS. Of the two oxygen functions, one was ascribed to a carbonyl group (δ_C 203.8) and one to a secondary hydroxy group (δ_C 65.1, δ_H 4.01) on the basis of ^{13}C and ^1H NMR data. The ^{13}C and ^1H NMR assignments for **1** are presented in the Experimental Section. Assignments were made on the basis of $^1H-^1H$ COSY, NOESY, HSQC, and HMBC experiments and by comparison with capsanthin.[10] The ^{13}C and ^1H NMR data of **1** were almost identical with those of capsanthin except for the signals of the end group (C1′ to C6′ including C16′, C17′, and C18′). The partial structures of a 3-hydroxy-β end group and all-*Z* polyene chain in **1** were confirmed from the 2D NMR spectra. The remaining end group consisted of nine carbons including three methyls (δ_C 20.9, 24.6, and 25.6), three methylenes (δ_C19.6, 34.4, and 40.5), and three quaternary carbons (δ_C 44.0, 58.9, and 203.8). The direct $^1H-^{13}C$ correlations of methyls and methylenes in this end group were assigned on the basis of the HSQC experiment. The ^1H signals of methylene protons in this end group except for H-4′α (δ_H 2.53) were overlapped with each other and showed second-order spin couplings. Therefore, the connections of protons from H-2′ to H-4′ were confirmed by the 1D TOCSY experiment. The HMBC spectrum revealed the following long-range $^1H-^{13}C$ correlations including quaternary carbons: H-16′/C-1′, C-2′, C-5′; H-17′/C-1′, C-2′, C-5′; H-18′/C-1′, C-4′, C-5′, C-6′; H-7′/C-6′; H-8′/C-6′. Furthermore, the NOESY spectrum showed the following NOE cross-peaks: H-16′/H-2′α; H-17′/H-2′β; H-17′/H-4′β; H-18′/H-2′β; H-4′α/H-4′β; H-17′/H-7′; H-18′/H-7′. The structure of the 6-oxo-κ end group was deduced on the basis of 1D TOCSY, HMBC, and NOESY experiments.

The positive ion FABCID-MS/MS spectrum of the molecular ion ($M^{+\bullet}$) of **1** at *m*/*z* 568 is shown in the Supporting Information. In addition to M-18$^{+\bullet}$ and M-92$^{+\bullet}$, the characteristic product ions of *m*/*z* 457 $[M - 111]^+$ (attributed to cleavage between C-6′ and C-5′) and *m*/*z* 429 $[M - 139]^+$ (attributed to cleavage between C-7′ and C-6′) were found. It was reported that capasnthin, possessing a 3-hydroxy-6-oxo-κ end group showed characteristic fragment ions resulting from cleavage between C-6′ and C-5′ at *m*/*z* 457 $[M - 127]^+$ and cleavage between C-7′ and C-6′ at *m*/*z* 429 $[M - 155]^+$ in EIMS[11] and FABMS/MS.[12] Therefore, the product ions of M − 111 and M − 139 in **1**, which were 16 mass units less than from the corresponding ions (M − 127 and M − 155) of capsanthin, clearly indicated the presence

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of a 6-oxo-κ end group. The CD spectrum of **1** was almost the same as that of capsanthin,[13] which has 3*R*, 3′*S*, 5′*R* chirality. On the basis of the CD data and biosynthetic considerations,[14] the 3*R*, 5′*R* chirality was postulated for **1**. Therefore, **1** was determined to be (3*R*,5′*R*)-3-hydroxy-β,κ-caroten-6′-one, and the compound was named 3′-deoxycapsanthin.

Compound **2** showed an absorption maximum at 475 nm without a fine structure. The molecular formula of **1** was determined to be $C_{40}H_{54}O$ by HRFABMS. Compound **2** also showed the characteristic product ions of M − 111$^+$ at *m*/*z* 439 and M − 139$^+$ at *m*/*z* 411, indicating the presence of a 6-oxo-κ end group, in the positive ion FABCID-MS/MS spectrum of the molecular ion at *m*/*z* 550 (Supporting Information). Meanwhile, the dehydroxy ion M − 18$^{+\bullet}$ was not observed. These results suggested that **2** was a dehydroxy derivative of **1**. The ^{1}H NMR including ^{1}H−^{1}H COSY and NOESY experiments revealed the presence of a 3,4-didehydro-β end group, a 6-oxo-κ end group, and an all-*Z* polyene chain in **2**. The CD spectrum of **2** was almost the same as that of capsorubin,[13] having 3*S*, 5*R*, 3′*S*, 5′*R* chilarity. On the basis of the CD data and biosynthetic considerations,[14] the 5′*R* chirality was postulated for **1**. Therefore the structure of **2** was deduced to be (5′*R*)-3,4-didehydro-β,κ-caroten-6′-one (3,4-dehydroxy-3′-deoxycapsanthin). These compounds are the first examples of carotenoids possessing a 6-oxo-κ end group.

Concerning the biosyntheses of carotenoids in paprika, capsanthin-capsorubin synthease (CCS), an enzyme catalyzing the conversion of a 5,6-epoxy end group into a 6-oxo-κ end group, was isolated from *C. annuum*.[15] Thus, 3′-deoxycapsanthin was assumed to be a product of the pinacollic rearrangement of β-cryptoxanthin-5′,6′-epoxide[16] catalyzed by CCS.

Experimental Section

General Experimental Procedures. The UV−vis spectra were recorded with a Shimadzu U-2001 spectrophotometer in diethyl ether (Et_2O). The positive ion FABMS and CIDMS/MS spectra were recorded using a JEOL JMS-HX/HX 110A four-sector tandem mass spectrometer with *m*-nitrobenzyl alcohol (*m*-NBA) as a matrix. The CIDMS/MS was performed with a FAB gun operated at 6 kV. A few micrograms of sample dissolved in benzene was placed on a stainless steel probe tip, and 1−2 μL of *m*-NBA was added as a matrix. The sample was bombarded with xenon atoms, and the ions produced were accelerated through 10 keV. 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 resulting product ions were acquired by linked scanning 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. Gradient (g) ^{1}H−^{1}H COSY, NOESY (mixing time 1.3 s), gHSQC ($^1J_{CH}$ = 142 Hz), gHMBC ($^nJ_{CH}$ optimized for 8 Hz), and 1D TOCSY (mixing time 0.05 s) spectra were aquired using the standard Varian pulse programs with the software Varian version 6.1A. The CD spectra were recorded in Et_2O at room temperature with a JASCO J-500 spectropolarimeter. HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer set at 450 nm.

Plant Material. Matured fruits were collected from paprika plants grown on a farm in Hitachinaka City, Ibaraki Prefecture, Japan.

Extraction and Isolation of Carotenoids. The MeOH extract of the fruits (4 kg) of *C. annuum* L. was partitioned between *n*-hexane−Et_2O (1:1) and aqueous NaCl. The organic layer was evaporated to dryness. The residue was saponified with 5% KOH−MeOH for 6 h at room temperature. Then unsaponifiable matter was extracted with *n*-hexane−Et_2O (1:1) and washed with H_2O. The organic layer was dried over Na_2SO_4 and then evaporated to dryness. The residue was subjected to silica gel column chromatography using an increasing percentage of Me_2CO in *n*-hexane. After the removal of steroids by filtration, the fraction eluted with Me_2CO−hexane (1:9) was subjected to a series of HPLCs on ODS with $CHCl_3$−MeCN (1:9) and on silica gel with Me_2CO−hexane (2:8) to yield **1** (2 mg) and **2** (0.5 mg).

3′-Deoxycapsanthin (1): UV−vis (Et_2O) λ_{max} 465 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.85 (3H, s, H-16′), 1.07 (6H, s, H-16, 17), 1.12 (3H, s, H-17′), 1.19 (3H, s, H-18′), 1.48 (1H, dd, *J* = 11.5, 11.5 Hz, H-2β), 1.49 (1H, m, H-4′β), 1.55 (1H, m, H-2′α), 1.68 (3H, m, H-2′β, H-3′α, H-3′β), 1.74 (3H, s, H-17), 1.77 (1H, ddd, *J* = 11.5, 4, 2 Hz, H-2α), 1.96 (3H, s, H-19′), 1.98 (6H, s, H-19, 20′), 1.99 (3H, s, H-20), 2.05 (1H, dd, *J* = 17, 9.5 Hz, H-4β), 2.39 (1H, ddd, *J* = 17, 5, 2 Hz, H-4α), 2.53 (1H, m, H-4′α), 4.01 (1H, m, H-3), 6.10 (1H, d, *J* = 16 Hz, H-7), 6.15 (1H, d, *J* = 16 Hz, H-8), 6.16 (1H, d, *J* = 11 Hz, H-10), 6.27 (1H, d, *J* = 11 Hz, H-14), 6.36 (1H, d, *J* = 11 Hz, H-14′), 6.37 (1H, d, *J* = 15.5 Hz, H-12), 6.48 (1H, d, *J* = 15 Hz, H-7′), 6.52 (1H, d, *J* = 15.5, H-12′), 6.55 (1H, d, *J* = 11 Hz, H-10′), 6.63 (1H, m, H-15′), 6.63 (1H, dd, *J* = 15.5, 11 Hz, H-11′), 6.68 (1H, dd, *J* = 15.5, 11 Hz, H-11), 6.69 (1H, m, H-15), 7.33 (1H, d, *J* = 15 Hz, H-8′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.8 (CH_3, C-19, 20, 19, 20′), 19.6 (CH_2, C-3′), 20.9 (CH_3, C-18′), 21.6 (CH_3, C-18), 24.6 (CH_3, C-17′), 25.6 (CH_3, C-16′), 28.7 (CH_3, C-16), 30.3 (CH_3, C-17), 34.4 (CH_2, C-4′), 37.1 (C, C-1), 40.5 (CH_2, C-2′), 42.6 (CH_2, C-4), 44.0 (C, C-1′), 48.4 (CH_2, C-2), 58.9 (C, C-5′), 65.1 (CH_2, C-3), 121.4 (CH, C-8′), 124.2 (CH, C-11′), 125.5 (CH, C-11), 125.8 (CH, C-7), 126.2 (C, C-5), 129.7 (CH, C-15′), 131.2 (CH, C-15), 131.5 (CH, C-10), 132.4 (CH, C-14), 133.8 (C, C-9′), 135.1 (CH, C-14′), 135.9 (C, C-9), 136.1 (C, C-13), 137.4 (C, C-6), 137.4 (CH, C-12), 137.7 (C, C-13′), 138.4 (CH, C-8), 140.3 (CH, C-12′), 141.7 (CH, C-10′), 146.4 (CH, C-7′), 203.8 (C, C-6′); CD (Et_2O) λ ($\Delta\epsilon$) 210 (0), 225 (−3.0), 232 (0), 252 (+3.1), 256 (0), 285 (−5.8), 315 (0), 340 (+2.0), 357 (0), 377 (−2.0); HRFABMS *m*/*z* 568.4287 (calcd for $C_{40}H_{56}O_2$, 568.4280).

3,4-Didehydroxy-3′-deoxycapsanthin (2): UV−vis (Et_2O) λ_{max} 475 nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.85 (3H, s, H-16′), 1.05 (6H, s, H-16, 17), 1.12 (3H, s, H-17′), 1.19 (3H, s, H-18′), 1.49 (1H, m, H-4′β), 1.55 (1H, m, H-2′α), 1.68 (3H, m, H-2′β, H-3′α, H-3′β), 1.88 (3H, s, H-17), 1.96 (3H, s, H-19′), 1.98 (6H, s, H-19, 20′), 1.99 (3H, s, H-20), 2.02 (2H, dd, *J* = 4.5, 1.5 Hz, H-2), 5.73 (1H, dt, *J* = 9.5, 4.5 Hz, H-3), 5.86 (1H, dt, *J* = 9.5, 1.5 Hz, H-4), 6.20 (1H, d, *J* = 11 Hz, H-10), 6.21 (1H, d, *J* = 16 Hz, H-7), 6.27 (1H, d, *J* = 11 Hz, H-14), 6.30 (1H, d, *J* = 16 Hz, H-8), 6.36 (1H, d, *J* = 11 Hz, H-14′), 6.37 (1H, d, *J* = 15.5 Hz, H-12), 6.48 (1H, d, *J* = 15 Hz, H-7′), 6.52 (1H, d, *J* = 15.5, H-12′), 6.55 (1H, d, *J* = 11 Hz, H-10′), 6.63 (1H, m, H-15′), 6.63 (1H, dd, *J* = 15.5, 11 Hz, H-11′), 6.67 (1H, m, H-15), 6.70 (1H, dd, *J* = 15.5, 11 Hz, H-11), 7.33 (1H, d, *J* = 15 Hz, H-8′); CD (Et_2O) λ ($\Delta\epsilon$) 250 (0), 260 (−0.4), 270 (0), 302 (+3.5), 320 (0), 365 (−2.1), 400 (+0.2); HRFABMS *m*/*z* 550.4172 (calcd for $C_{40}H_{54}O$, 550.4175).

Capsanthin: CD (Et_2O) λ ($\Delta\epsilon$) 210 (0), 220 (−6.3), 232 (0), 250 (+7.0), 265 (0), 275 (−4.2), 290 (−8.5), 318 (0), 350 (+1.7), 368 (0).

Capsorubin: CD (Et_2O) λ ($\Delta\epsilon$) 235 (0), 240 (−3.2), 260 (0), 300 (+4.6), 310 (0), 360 (−4.3), 380 (−1.5).

Supporting Information Available: FABMS/MS of M$^{+\bullet}$ of 3′-deoxycapsanthin (**1**) and 3,4-dehydroxy-3′-deoxycapsanthin (**2**). This material is available free charge via the Internet at http://pubs.acs.org.

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(16) β-Cryptoxanthin-5′,8′-epoxide, which was the corresponding product of the epoxy-furanoxide rearrangement of β-cryptoxanthin-5′,6′-epoxide, was isolated from *C. annuum* in the present study instead of β-cryptoxanthin-5′,6′-epoxide.

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