

# Isolation of a Series of Apocarotenoids from the Fruits of the Red Paprika *Capsicum annuum* L.

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Eleven apocarotenoids (**1–11**) including five new compounds, **4**, **6**, **9**, **10** and **11**, were isolated from the fruits of the red paprika *Capsicum annuum* L. The structures of new apocarotenoids were determined to be apo-14'-zeaxanthinal (**4**), apo-13'-zeaxanthinone (**6**), apo-12'-capsorubinal (**9**), apo-8'-capsorubinal (**10**), and 9,9'-diapo-10,9'-retro-carotene-9,9'-dione (**11**) by spectroscopic analysis. The other six known apocarotenoids were identified to be apo-8'-zeaxanthinal (**1**), apo-10'-zeaxanthinal (**2**), apo-12'-zeaxanthinal (**3**), apo-15'-zeaxanthinal (**5**), apo-11'-zeaxanthinal (**7**), and apo-9'-zeaxanthinone (**8**) which have not been previously found in paprika. These apocarotenoids were assumed to be oxidative cleavage products of C<sub>40</sub> carotenoid such as capsanthin in paprika.

**Keywords:** *Capsicum annuum*; apocarotenoids; apo-14'-zeaxanthinal; apo-13'-zeaxanthinone; apo-8'-capsorubinal; apo-12'-capsorubinal; 9,9'-diapo-10,9'-retro-carotene-9,9'-dione

## INTRODUCTION

Ripe fruits of paprika (red pepper) are used widely as vegetables and food colorants, which are good source of carotenoid pigments. The red carotenoids in paprika (*Capsicum annuum* L.) are mainly capsanthin, capsorubin, and capsanthin 5,6-epoxide which are peculiar to this genus (*1, 2, 3*). At the same time, the fruits are also rich in yellow xanthophylls such as  $\beta$ -cryptoxanthin, zeaxanthin, violaxanthin, antheraxanthin, and  $\beta$ -carotene (*4, 5*). Furthermore, many other carotenoids with interesting structures, especially those with the 3,5,6-trihydroxy-5,6-dihydro- $\beta$ -end group (karpoxanthin) (*6*), 3,4-didehydro-6-hydroxy- $\gamma$ -end group (nigroxanthin) (*7*), and 5-hydroxy-5,6-didehydro-3,6-epoxy- $\beta$ - (oxabicyclo) end group (cycloviolaxanthin, cucurbitaxanthins, and capsanthin 3,6-epoxide) (*8, 9, 10, 11*) have been isolated.

The biosynthesis pathways of carotenoids with a 3-hydroxy- $\kappa$ -end group such as capsanthin and capsorubin and a 5-hydroxy-3,6-epoxy end group such as cucurbitaxanthin A and capsanthin 3,6-epoxide in paprika during fruit ripening were reported by Davies et al. (*1*), Deli et al. (*11*), and Hornero-Mendezu et al. (*12, 13*).

In the course of our studies on paprika carotenoids, 11 apocarotenoids (**1–11**), including five new compounds **4**, **6**, **9**, **10**, and **11**, were isolated from the fruits of the red paprika *C. annuum* L. as minor components along with 18 known C<sub>40</sub> carotenoids. This paper reports the isolation and the structural elucidation of these apocarotenoids. Furthermore, possible routes for formation of these apocarotenoids from C<sub>40</sub> carotenoid were discussed.

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## MATERIALS AND METHODS

**Apparatus.** The UV–visible (vis) spectra was recorded with a Shimadzu UV-240 spectrophotometer. The electron impact mass spectra (EI-MS) were recorded using a JEOL JMS-HX/HX 110A mass spectrometer. The <sup>1</sup>H NMR (500 MHz) and <sup>13</sup>C NMR (125 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer in CDCl<sub>3</sub> with TMS as an internal standard. DQF-COSY, NOESY (mixing time 1.3 s), gHSQC (<sup>1</sup>J<sub>CH</sub> = 142 Hz) and gHMBC (<sup>n</sup>J<sub>CH</sub> optimized for 8 Hz) spectra were acquired using the standard Varian pulse programs, and the software used to obtain 2D spectra was from Varian, version 6.1A. The CD spectra were recorded in ether (Et<sub>2</sub>O) at room temperature with a JASCO J-500 spectropolarimeter. HPLC was performed on a Shimadzu LC-6AD instrument with a Shimadzu SPD-6AV spectrophotometer set at 380 nm or 280 nm. The column used was a Lichrospher 100 RP-18 (Cica Merck, 20 mm × 250 mm, 10  $\mu$ m) using dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>)–acetonitrile (CH<sub>3</sub>CN) (5:95) as the mobile phase, flow rate of 5 mL/min.

**Plant Material.** The matured fruits were collected from paprika plants grown at the farm in Hitachinaka city, Ibaraki Prefecture, Japan.

**Extraction and Isolation of Carotenoids.** The methanol (MeOH) extract of the fruits (800 g) of *C. annuum* L. was partitioned between *n*-hexane–Et<sub>2</sub>O (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<sub>2</sub>O (1:1) and washed with water. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and then concentrated to dryness. The residue was subjected to silica gel column chromatography using an increasing percentage of acetone (Me<sub>2</sub>CO) in *n*-hexane. A series of apocarotenoids were eluted with Me<sub>2</sub>CO–*n*-hexane (2:8) from a silica gel column and further purified by HPLC on a C<sub>18</sub> reversed phase column with CH<sub>2</sub>Cl<sub>2</sub>–CH<sub>3</sub>CN (5:95) as the solvent.

**Apo-8'-zeaxanthinal (1):** yield 1 mg (0.4% of the total carotenoid); retention time on HPLC (*R*<sub>t</sub>) 9.2 min; UV–vis  $\lambda$  max (Et<sub>2</sub>O) nm 456; high resolution (HR) EI-MS *m/z* [M<sup>+</sup>] 432.3024 (C<sub>30</sub>H<sub>40</sub>O<sub>2</sub> calcd 432.3029); EI-MS (70 eV) *m/z* (rel int, %) 432 [M<sup>+</sup>] (100), 414 (10), 119 (17), 105 (15), 91 (12); CD (Et<sub>2</sub>O)  $\lambda$  nm ( $\Delta\epsilon$ ) 239 (2), 250 (0), 272 (–5); <sup>1</sup>H NMR, the chemical shift and spin coupling constant values were in agreement with previously published values (*14*).

**Table 1.**  $^1\text{H}$  NMR of Apocarotenoids **3a**, **3b**, **4a**, **4b**, **5**, **6a**, **6b**, **9**, **10**, and **11** in  $\text{CDCl}_3^a$ 

position	<b>3a</b>	<b>3b</b>	<b>4a</b>	<b>4b</b>	<b>6a</b>	<b>6b</b>	<b>9</b>	<b>10</b>	<b>11</b>
H-2	1.48 dd (12, 12) 1.77 ddd (12, 3, 1.5)	1.48 dd (12, 12) 1.77 ddd (12, 3, 1.5)	1.48 dd (12, 12) 1.77 ddd (12, 3, 1.5)	1.48 dd (12, 12) 1.77 ddd (12, 3, 1.5)	1.48 dd (12, 12) 1.77 ddd (12, 3, 1.5)	1.48 dd (12, 12) 1.77 ddd (12, 3, 1.5)	1.77 dd (14, 3) 2.00 dd (17, 8)	1.77 dd (14, 3) 2.00 dd (17, 8)	
H-3	4.00 m	4.00 m	4.00 m	4.00 m	4.00 m	4.00 m	4.52 m	4.52 m	
H-4	2.04 dd (16, 10) 2.39 ddd (16, 5.5, 1.5)	2.04 dd (16, 10) 2.39 ddd (16, 5.5, 1.5)	2.04 dd (16, 10) 2.39 ddd (16, 5.5, 1.5)	2.04 dd (16, 10) 2.39 ddd (16, 5.5, 1.5)	2.04 dd (16, 10) 2.39 ddd (16, 5.5, 1.5)	2.04 dd (16, 10) 2.39 ddd (16, 5.5, 1.5)	1.49 dd (14, 3) 2.96 dd (14, 8)	1.49 dd (14, 3) 2.96 dd (14, 8)	
H-7	6.18 d (17)	6.18 d (17)	6.18 d (16)	6.18 d (16)	6.35 d (15.5)	6.32 d (15.5)	6.49 d (15)	6.49 d (15)	
H-8	6.16 d (17)	6.16 d (17)	6.16 d (16)	6.16 d (16)	6.17 d (15.5)	6.71 d (15.5)	7.32 d (15)	7.32 d (15)	
H-10	6.17 d (11)	6.20 d (11)	6.17 d (11)	6.22 d (11)	6.17 d (11.5)	6.09 d (11.5)	6.56 d (11.5)	6.56 d (11.5)	6.22 d (15)
H-11	6.79 dd (15, 11)	6.78 dd (15, 11)	6.89 dd (15, 11)	~6.91	7.56 dd (15, 11.5)	7.63 dd (15, 11.5)	6.75 dd (15, 11.5)	6.67 dd (15, 11.5)	7.54 dd (15, 11.5)
H-12	6.38 d (15)	6.91 d (15)	6.39 d (15)	~6.91	6.18 d (15)	6.13 d (11.5)	6.53 d (15)	6.52 d (15)	6.27 d (11.5)
H-14	6.31 d (12)	6.17 d (12)	6.36 d (12)	6.22 d (12)			6.39 d (15)	6.37 d (15)	6.56* (15)
H-15	7.03 dd (15, 12)	7.19 dd (15, 12)	7.52 dd (15, 12)	7.66 dd (15, 12)			7.03 dd (14, 11)	7.03 dd (14, 11)	6.50*
H-15'	6.69 dd (15, 12)	6.63 dd (15, 8)	6.18 dd (15, 8)	6.12 dd (15, 8)			6.75 dd (14, 11.5)	6.67 d (14, 11.5)	6.50*
H-14'	6.96 d (12)	6.95 d (12)	9.61 d (8)	9.61 d (8)			6.96 d (11.5)	6.45 d (11.5)	6.56*
H-12'	9.46 s	9.46 s					9.44 s	6.72 d (15)	6.27 d (11.5)
H-11'								6.67 dd (15, 11.5)	7.54 dd (15, 11.5)
H-10'								6.95 d (11.5)	6.22 d (15)
H-8'								9.46 s	
H <sub>3</sub> -16	1.08 s	1.08 s	1.08 s	1.08 s	1.08 s	1.08 s	0.84 s	0.84 s	
H <sub>3</sub> -17	1.08 s	1.08 s	1.08 s	1.08 s	1.08 s	1.08 s	1.21 s	1.21 s	
H <sub>3</sub> -18	1.74 s	1.74 s	1.74 s	1.74 s	1.74 s	1.74 s	1.37 s	1.37 s	
H <sub>3</sub> -19	1.93 s	2.00 s	2.03 s	2.03 s	2.06 s	2.04 s	1.98 s	1.97 s	2.31 s
H <sub>3</sub> -20	2.05 s	2.06 s	2.13 s	2.12 s	2.30 s	2.29s	2.05 s	2.01 s	2.06 s
H <sub>3</sub> -20'	1.89 s	1.88 s					1.90 s	2.01 s	2.06 s
H <sub>3</sub> -19'								1.91 s	2.31 s

<sup>a</sup>  $\delta$  values in  $\text{CDCl}_3$  at 500 MHz. Values in parentheses are coupling constants (Hz). <sup>b</sup> Asterisk indicated an AA'BB' spin system.

**Apo-10'-zeaxanthinal (2):** yield 1 mg (0.4% of the total carotenoid);  $R_t$  7.2 min; UV-vis  $\lambda$  max ( $\text{Et}_2\text{O}$ ) nm 436; HR EI-MS  $m/z$  [ $\text{M}^+$ ] 392.2713 ( $\text{C}_{27}\text{H}_{36}\text{O}_2$  calcd 392.2715); EI-MS (70 eV)  $m/z$  (rel int, %) 392 [ $\text{M}^+$ ] (100), 374 (10), 119 (20), 105 (18), 91 (15); CD ( $\text{Et}_2\text{O}$ )  $\lambda$  nm ( $\Delta\epsilon$ ) 225 (+0.5), 250 (-3), 275 (-0.5), 315 (-3), 340 (0), 370 (-1);  $^1\text{H}$  NMR, the chemical shift values of methyl signals of **2** were in agreement with previously published values (15).

**Apo-12'-zeaxanthinal (3):** yield 1 mg (0.4% of the total carotenoid);  $R_t$  6.8 min, UV-vis  $\lambda$  max ( $\text{Et}_2\text{O}$ ) nm 420; HR EI-MS  $m/z$  [ $\text{M}^+$ ] 366.2565 ( $\text{C}_{25}\text{H}_{34}\text{O}_2$  calcd 366.2571); EI-MS (70 eV)  $m/z$  (rel int, %) 366 [ $\text{M}^+$ ] (100), 348 (15), 119 (25), 105 (25), 91 (25); CD ( $\text{Et}_2\text{O}$ )  $\lambda$  nm ( $\Delta\epsilon$ ) 213 (-0.5), 251 (+0.5), 305 (-2.6), 330 (0);  $^1\text{H}$  NMR see Table 1.  $^1\text{H}$  NMR revealed the presence of *all-E* (**3a**) and 13-*Z* (**3b**) isomers, and the ratio of **3a** and **3b** was determined to be 3:1. The chemical shift and spin coupling values of **3a** were in agreement with previously published values (16).

**Apo-14'-zeaxanthinal (4):** yield 0.5 mg (0.2% of the total carotenoid);  $R_t$  5.5 min; UV-vis  $\lambda$  max ( $\text{Et}_2\text{O}$ ) nm 400; HR EI-MS  $m/z$  [ $\text{M}^+$ ] 326.2241 ( $\text{C}_{22}\text{H}_{30}\text{O}_2$  calcd 326.2246); EI-MS (70 eV)  $m/z$  (rel int, %) 326 [ $\text{M}^+$ ] (100), 308 (10), 119 (45), 105 (55), 91 (53); CD ( $\text{Et}_2\text{O}$ )  $\lambda$  nm ( $\Delta\epsilon$ ) 230 (-0.5), 280 (+0.1), 300 (-1.6), 350 (0.5);  $^1\text{H}$  NMR see Table 1.  $^1\text{H}$  NMR revealed the presence of *all-E* (**4a**) and 13-*Z* (**4b**) isomers, and the ratio of **4a** and **4b** was determined to be 3:2.

**Apo-15-zeaxanthinal (5):** yield 0.1 mg (0.04% of the total carotenoid);  $R_t$  5.0 min; UV-vis  $\lambda$  max ( $\text{Et}_2\text{O}$ ) nm 378; HR EI-MS  $m/z$  [ $\text{M}^+$ ] 300.2091 ( $\text{C}_{20}\text{H}_{28}\text{O}_2$  calcd 300.2089); EI-MS (70 eV)  $m/z$  (rel int, %) 300 [ $\text{M}^+$ ] (100), 119 (75), 105 (73), 91 (70); CD ( $\text{Et}_2\text{O}$ )  $\lambda$  nm ( $\Delta\epsilon$ ) 220 (0), 250 (-2), 290 (0), 375 (+2);  $^1\text{H}$

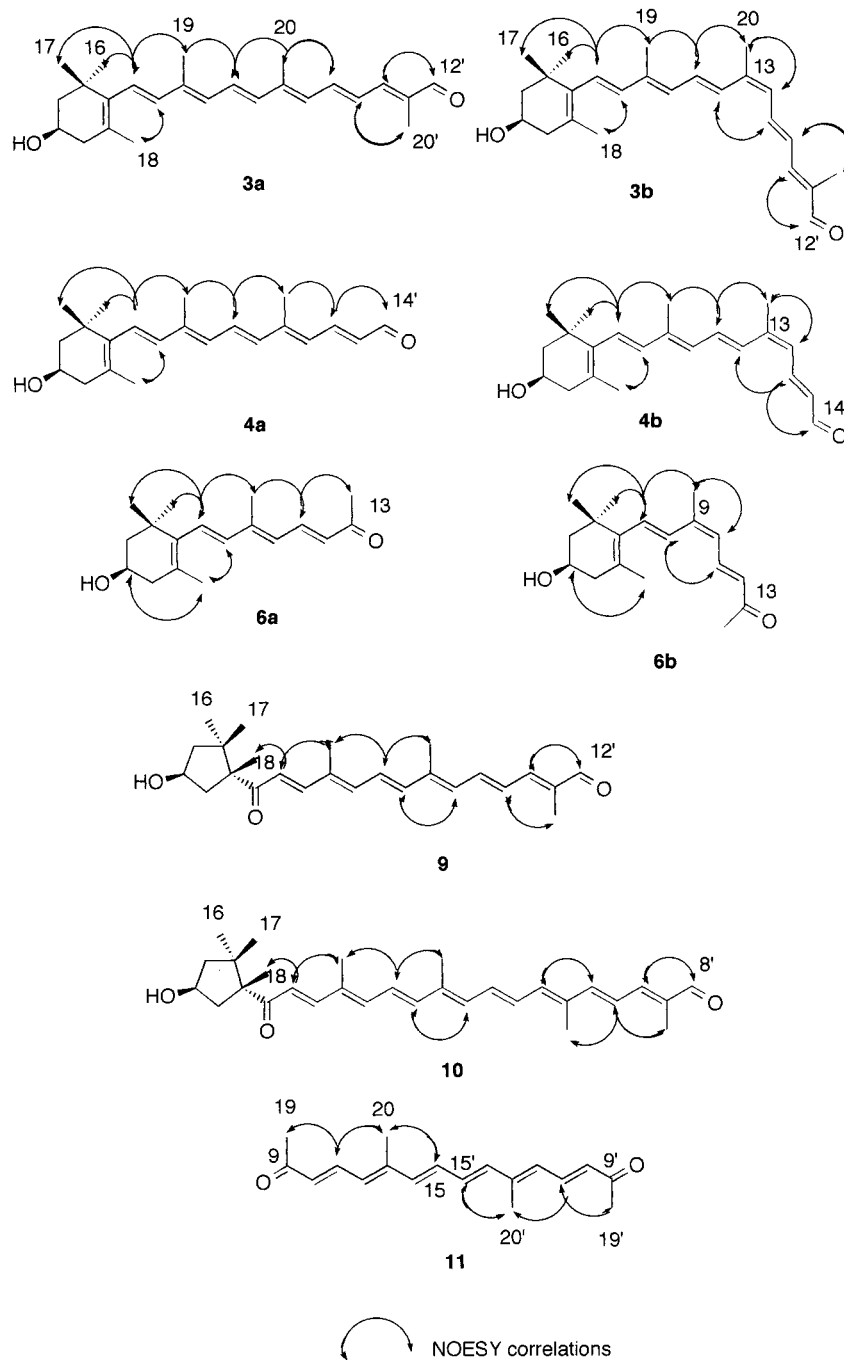
NMR showed the presence of minor geometrical isomers together with *all-E* isomer. The chemical shift values and spin coupling constant values of *all-E* isomer were in agreement with previously published values (17).

**Apo-13-zeaxanthinone (6):** yield 2 mg (0.8% of the total carotenoid);  $R_t$  4.5 min, UV-vis  $\lambda$  max ( $\text{Et}_2\text{O}$ ) nm 338; HR EI-MS  $m/z$  [ $\text{M}^+$ ] 274.1932 ( $\text{C}_{18}\text{H}_{26}\text{O}_2$  calcd 274.1933); EI-MS (70 eV)  $m/z$  (rel int, %) 274 [ $\text{M}^+$ ] (85), 241 (60), 78 (50), 43 (100); CD ( $\text{Et}_2\text{O}$ )  $\lambda$  nm ( $\Delta\epsilon$ ) 220 (-2), 280 (0), 285 (+0.2), 330 (-1.0), 380 (0);  $^1\text{H}$  NMR see Table 1.  $^1\text{H}$  NMR revealed the presence of *all-E* (**6a**) and 9-*Z* (**6b**) isomers, and the ratio of **6a** and **6b** was determined to be 4:1.  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  13.1 (C-19), 21.6 (C-18), 27.7 (C-20), 28.7 (C-16), 30.2 (C-17), 37.1 (C-1), 42.5 (C-4), 48.4 (C-2), 64.9 (C-3), 127.6 (C-5), 128.1 (C-10)\*, 129.6 (C-12), 130.2 (C-7), 137.3 (C-6), 137.5 (C-8)\*, 139.1 (C-11), 145.1 (C-9), 198.3 (C-13). An asterisk indicates that assignments may be interchanged.

**Apo-11-zeaxanthinal (7):** yield 0.5 mg (0.2% of the total carotenoid);  $R_t$  4.0 min, UV-vis  $\lambda$  max ( $\text{Et}_2\text{O}$ ) 300 nm; HR EI-MS  $m/z$  [ $\text{M}^+$ ] 234.1614 ( $\text{C}_{15}\text{H}_{22}\text{O}_2$  calcd 234.1619); EI-MS (70 eV)  $m/z$  (rel int, %) 234 [ $\text{M}^+$ ] (70), 216 (20), 95 (100), 43 (75);  $^1\text{H}$  NMR see Table 1. These spectral data of **7** were in agreement with previously published values (18).

**Apo-9-zeaxanthinone (8):** yield 0.2 mg (0.08% of the total carotenoid);  $R_t$  3.8 min, UV-vis  $\lambda$  max ( $\text{Et}_2\text{O}$ ) nm 220, 285; HR EI-MS  $m/z$  [ $\text{M}^+$ ] 208.1470 ( $\text{C}_{13}\text{H}_{20}\text{O}_2$  calcd 208.1435); EI-MS (70 eV)  $m/z$  (rel int, %) 208 [ $\text{M}^+$ ] (85), 190 (5), 78 (50), 43 (100);  $^1\text{H}$  NMR see Table 1.

**Apo-12'-capsorubinal (9):** yield 0.5 mg (0.2% of the total carotenoid);  $R_t$  5.5 min; UV-vis  $\lambda$  max ( $\text{Et}_2\text{O}$ ) nm 313, 413, 437; HR EI-MS  $m/z$  [ $\text{M}^+$ ] 382.2511 ( $\text{C}_{25}\text{H}_{34}\text{O}_3$  calcd 382.2508);



**Figure 1.** Structures and key NOESY correlations of apocarotenoids **3a**, **3b**, **4a**, **4b**, **6a**, **6b**, **9**, **10**, and **11**.

EI-MS (70 eV)  $m/z$  (rel int, %) 382 [ $M^+$ ] (100), 364 (5), 255 (12), 229 (10), 227 (11), 159 (30), 109 (60), 83 (35); CD (Et<sub>2</sub>O)  $\lambda$  nm ( $\Delta\epsilon$ ) 250 (+0.5), 280 (0), 335 (+1), 356 (0); <sup>1</sup>H NMR see Table 1.

**Apo-8'-capsorubinal (10):** yield 0.2 mg (0.08% of the total carotenoid);  $R_t$  7.6 min; UV-vis  $\lambda$  max (Et<sub>2</sub>O) nm 428, 458, 485; HR EI-MS  $m/z$  [ $M^+$ ] 448.2972 (C<sub>30</sub>H<sub>40</sub>O<sub>3</sub> calcd 448.2978); EI-MS (70 eV)  $m/z$  (rel int, %) 448 [ $M^+$ ] (100), 430 (5), 321 (8), 295 (4), 227 (5), 145 (45), 109 (75), 83(45); CD (Et<sub>2</sub>O)  $\lambda$  nm ( $\Delta\epsilon$ ) 230 (+0.7), 260 (0), 320 (+2), 340 (0), 350 (-0.8); <sup>1</sup>H NMR see Table 1.

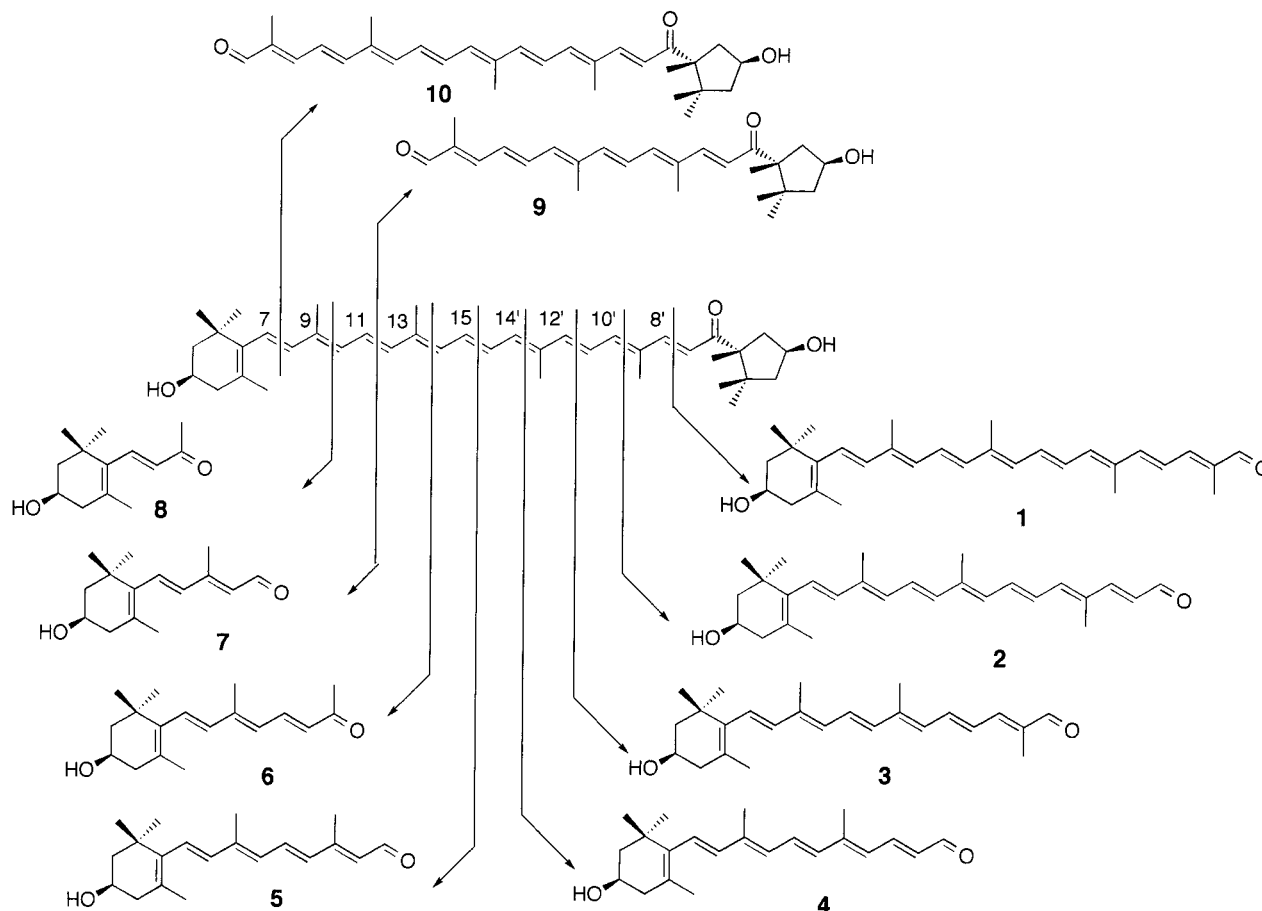
**9,9'-Diapo-10,9'-retro-carotene-9,9'-dione (11):** yield 2 mg (0.8% of the total carotenoid);  $R_t$  4.3 min; UV-vis  $\lambda$  max (Et<sub>2</sub>O) nm 410, 432; HR EI-MS  $m/z$  [ $M^+$ ] 270.1622 (C<sub>18</sub>H<sub>22</sub>O<sub>2</sub> calcd 270.1620); EI-MS (70 eV)  $m/z$  (rel int, %) 270 [ $M^+$ ] (75), 225 (8), 227 (45), 185 (30), 169 (35), 109 (45), 91 (35), 78 (33), 43 (100); <sup>1</sup>H NMR see Table 1.

The following additional carotenoids were identified from the matured fruits of the red paprika *C. annuum*:  $\beta$ -carotene

(10 mg, 4% of total carotenoid),  $\beta$ -cryptoxanthin (8 mg, 3%),  $\alpha$ -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%), and neoxanthin (1 mg, 0.1%). They were identified by UV-vis, EI-MS, <sup>1</sup>H NMR, and CD spectral data.

## RESULTS AND DISCUSSION

The MeOH extract of the matured fruits of *C. annuum* (800 g) was saponified with 5% KOH/MeOH, and unsaponifiable matter was chromatographed on silica gel using an increasing percentage of Me<sub>2</sub>CO in *n*-



**Figure 2.** Possible routes for formation of apocarotenoids by oxidative cleavage of double bonds in the polyene chain in capsanthin.

hexane. Successive purification by 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 series of apocarotenoids (1–11).

Apocarotenoids 1, 2, 3, 5, 7, and 8 were identified to be apo-8'-zeaxanthinal ( $\beta$ -citraurine), apo-10'-zeaxanthinal, apo-12'-zeaxanthinal, apo-15-zeaxanthinal (3-hydroxyretinal), apo-11-zeaxanthinal (3-hydroxy- $\beta$ -ionylidene acetaldehyde), and apo-9-zeaxanthinone (3-hydroxy- $\beta$ -ionone), respectively, by UV-vis, EI-MS and  $^1H$  NMR data (14, 15, 16, 17, 18, 19).

Detailed  $^1H$  NMR studies including 2D NMR experiments for 3 revealed that 3 was a mixture of *all-E* (3a) and 13-*Z* (3b) isomers. The stereostructures of each geometrical isomers were confirmed by DQF-COSY and NOESY experiments as shown in Figure 1. Compound 5 was also obtained as a mixture of geometrical isomers. The major geometrical isomer was identified to be *all-E* by  $^1H$  NMR data (17). However, other minor isomers could not be identified because of the small amount of product. The five known apocarotenoids described above had not been previously found in paprika.

Compound 4 showed an absorption maximum at 400 nm, suggesting the presence of an apo-14'- $\beta$ -carotenal type chromophore (20, 21). HR EI-MS established the molecular formula  $C_{22}H_{30}O_2$ . Of the two oxygen functions, one was ascribed to a secondary hydroxy group ( $\delta_H$  4.00) and of the remaining one was attributed to an aldehyde group ( $\delta_C$  9.61) by  $^1H$  NMR data (Table 1). The partial structure of the 3-hydroxy- $\beta$ -end group and the polyene chain in 4 were characterized by  $^1H$  NMR, including DQF-COSY and NOESY experiments. From the spectral data described above, the structure of 4 was

deduced to be 3-hydroxy-14'-apo- $\beta$ -caroten-14'-al and was designated apo-14'-zeaxanthinal. Detailed  $^1H$  NMR studies including 2D NMR experiments of 4 revealed the presence of *all-E* (4a) and 13-*Z* (4b) geometrical isomers, and the ratio of 4a and 4b was determined to be 3:2 by the intensity of the corresponding  $^1H$  NMR signals. The *all-E* geometry for 4a was confirmed by NOESY correlations  $CH_3$ -19/H-7 and H-11,  $CH_3$ -20/H-11 and H-15, and H-15/H-14'. On the other hand, the 13-*Z* geometry for 4b was deduced by NOESY correlations  $CH_3$ -20/H-14 and H-15/H-12 as shown in Figure 1.

Compound 6 showed an absorption maximum at 338 nm, suggesting the presence of an apo-13- $\beta$ -carotenal type chromophore (20, 21). The molecular formula was determined to be  $C_{18}H_{26}O_2$  by HR EI-MS. Of the two oxygen functions, one was ascribed to a secondary hydroxy group ( $\delta_H$  4.00,  $\delta_C$  64.9) and the remaining one was attributed to a carbonyl group ( $\delta_C$  198.3) by  $^1H$  NMR and  $^{13}C$  NMR data. The partial structure of the 3-hydroxy- $\beta$ -end group and the polyene chain in 6 were characterized by  $^1H$  NMR (Table 1) and  $^{13}C$  NMR including DQF-COSY, NOESY, HSQC, and HMBC experiments. NOESY correlations  $CH_3$ -16/H-3 and H-7,  $CH_3$ -17/H-7,  $CH_3$ -18/H-3, and  $CH_3$ -19/H-7 and H-11 revealed the stereostructure of 6a as shown in Figure 1. Therefore, the structure of 6 was deduced to be 3-hydroxy-13-apo- $\beta$ -caroten-13-one and was designated apo-13-zeaxanthinone. The  $^1H$  NMR of 6 also showed the presence of 9-*Z* isomer 6b as a minor component. The ratio of *all-E* (6a) and 9-*Z* (6b) was determined to be 4:1 by the intensity of the corresponding  $^1H$  NMR signals. NOESY correlations  $CH_3$ -19/H-10 and H-8/H-



**11** in **6b** confirmed the 9-*Z* geometry as shown in Figure 1.

Compound **9** showed absorption maxima at 313, 413, and 437 nm, suggesting the presence of an octaene system (20). The molecular formula was determined to be C<sub>25</sub>H<sub>34</sub>O<sub>3</sub> by HR EI-MS. The partial structure of the 3-hydroxy- $\kappa$ -end group and the polyene chain in **9** were characterized by <sup>1</sup>H NMR (Table 1) including DQF-COSY and NOESY experiments. NOESY correlations CH<sub>3</sub>-18/H-7, CH<sub>3</sub>-19/H-7 and H-11, CH<sub>3</sub>-20/H-11 and H-15, CH<sub>3</sub>-20'/H-15', H-12/H-14, and H-12'/H-14' revealed the *all-E* geometry of double bonds in the polyene chain as shown in Figure. 1. From the spectral data described above, the structure of **9** was deduced to be 3-hydroxy-12'-apo- $\kappa$ -caroten-12'-al and was designated apo-12'-capsorubinal.

Compound **10** showed absorption maxima at 428, 458, and 485 nm, suggesting the presence of a decaene system (20). The molecular formula was determined to be C<sub>30</sub>H<sub>40</sub>O<sub>3</sub> by HR EI-MS. Similarly, the partial structure of the 3-hydroxy- $\kappa$ -end group and the polyene chain in **10** were also characterized by <sup>1</sup>H NMR (Table 1) including 2D NMR experiments. From the spectral data described above, the structure of **10** was deduced to be 3-hydroxy-8'-apo- $\kappa$ -caroten-8'-al and was designated apo-8'-capsorubinal (Figure 1).

The absolute stereochemistry of new apocarotenoids described above (**1–10**) was tentatively postulated by NOESY and CD (22) spectral data and consideration of the absolute configuration of capsanthin which was assumed to be a precursor of these apocarotenoids (Figure 2).

Compound **11** showed absorption maxima at 410 and 432 nm, suggesting the presence of an octaene system (20). The molecular formula was determined to be C<sub>18</sub>H<sub>22</sub>O<sub>2</sub> by HR EI-MS. <sup>1</sup>H NMR (Table 1) exhibited signals of two methyl groups and five olefinic protons, indicating a symmetrical structure. The downfield-shifted methyl signal at  $\delta$  2.31 indicated the presence of a methyl ketone group in **11** (19). The DQF-COSY experiment revealed the following proton-proton connectivities: H-10 (10') to H-12 (12'), H-14 (14') to H-15 (15'), and CH<sub>3</sub>-20 (20') to H-12 (12'). The methyl signal at  $\delta$  2.06 showing long-range coupling with H-12 (12') and NOESY correlations to H-15 (15') and H-11 (11') was assigned to be CH<sub>3</sub>-20 (20') and the other methyl signal at  $\delta$  2.31 exhibiting NOESY correlation to H-11 (11') was deduced to be CH<sub>3</sub>-19 (19'). The long-range coupling between CH<sub>3</sub>-20 (20') and H12 (12') was consistent with the presence of *retro* type double bonds in the polyene chain (23). The *all-E* geometry was confirmed by NOESY correlations as shown in Figure 1. Thus, the structure of **11** was determined to be 9,9'-diapo-10,9'-*retro*-carotene-9,9'-dione and was designated 9,9'-diapo-*retro*-carotene-9,9'-dione.

Contents and percentage composition of C<sub>40</sub> carotenoids identified from *C. annuum* L. in this study are compiled in the Materials and Methods section. They showed almost identical carotenoids patterns to those previously reported in *C. annuum* L. (3, 4, 5) except for the disappearance of capsanthin 5,6-epoxide.

More than 60 types of apocarotenoids have been found in various higher plants (24, 25), and most of them are assumed to be oxidative cleavage products of C<sub>40</sub> carotenoids (26).

Recently, Wu et al. (21) reported the formation of a number of apocarotenoids, including apocarotenal, ep-

oxycarotenal, apocarotene, and epoxy-carotene, by lipoxygenase-catalyzed co-oxidation of  $\beta$ -carotene. Therefore, a series of apocarotenoids (**1–10**) were also assumed to be oxidative cleavage products of C<sub>40</sub> carotenoid such as capsanthin in paprika. The possible routes for formation of apocarotenoids **1–10** by oxidative cleavage of the double bonds in the polyene chain in capsanthin are shown in Figure 2. 9,9'-Diapo-*retro*-carotene-9,9'-dione (**11**) was also assumed to be an oxidative cleavage product of C<sub>40</sub> carotenoid. However, the mechanisms of cleavages at C(8)–C(9) and C(8')–C(9') and *retro* rearrangement of double bonds in the polyene chain were uncertain.

In summary, a series of apocarotenoids possessing the 3-hydroxy- $\beta$ -end group (**1–8**) and the 3-hydroxy- $\kappa$ -end group (**9** and **10**) and 9,9'-diapo-*retro*-carotene-9,9'-dione (**11**) were isolated from the matured fruits of red paprika and were characterized by spectroscopic data. These apocarotenoids were assumed to be oxidative cleavage products of capsanthin.

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