Products Formed by Peroxyl Radical-Mediated Oxidation of Canthaxanthin in Benzene and in Methyl Linoleate

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Canthaxanthin was reacted with peroxyl radicals generated by thermolysis of 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) in benzene. The reaction products were separated by reversed-phase high-performance liquid chromatography, and their structures were identified as a mixture of 12-formyl-11-nor-β,β-carotene-4,4′-dione (**1a**) and 15′-formyl-15-nor-β,β-carotene-4,4′-dione (**1b**), 15′,13-epoxyvinyleno-13,15′-dihydro-14,15-dinor-β,β-carotene-4,4′-dione (**2**), 13,15′-epoxyvinyleno-13,15′-dihydro-14,15-dinor-β,β-carotene-4,4′-dione (**3**), and 11,15′-dihydrooxepino-β,β-carotene-4,4′-dione (**4**). Compounds **1**−**4** could be accounted for almost all the consumed canthaxanthin at an early stage of the reaction mixture. The peroxidation of methyl linoleate initiated by AMVN in bulk phase was suppressed by the addition of canthaxanthin. The reaction products of canthaxanthin, **1**−**4**, were also formed during the antioxidant process of canthaxanthin, although their yields were low. The result indicates that the peroxyl radical addition occurred in the antioxidant process of canthaxanthin during the AMVN-initiated peroxidation of methyl linoleate.

Keywords: *Canthaxanthin; carotenoids; lipid peroxidation; antioxidant; free radical*

INTRODUCTION

Carotenoids are widely distributed in nature, where they play an important role in protecting cells and organisms. They are extremely efficient quenchers of singlet oxygen which is generated by photochemical reactions (Foote, 1976). In addition, carotenoids may act as free radical-trapping antioxidants (Krinsky, 1993; Edge et al., 1997). It has been proposed that β-carotene acts as an antioxidant by an addition reaction to the double bond to give resonance-stabilized, carbon-centered, conjugated radicals (Burton and Ingold, 1984). Many studies have shown that carotenoids exhibit a protective effect against lipid peroxidation mediated by free radicals, in organic solution, liposomes, liver microsomes, and cells in culture (Terao, 1989; Miki, 1991; Kennedy and Liebler, 1992; Lim et al., 1992; Palozza and Krinsky, 1992; Tsuchihashi et al., 1995; Palozza et al., 1996; Carpenter et al., 1997; Woodall et al., 1997b; Yamauchi et al., 1998).

The fundamental chemistry of the reactions of carotenoids with free radicals is important for evaluating the proposed action of carotenoids as free radical scavengers. Thus, the products formed during the autoxidation or peroxyl radical-mediated oxidation of β-carotene have been investigated (Kennedy and Liebler, 1991; Handelman et al., 1991; Mordi et al., 1993; Yamauchi et al., 1993; Liebler and McClure, 1996; Woodall et al., 1997a). The oxidation products of β-carotene with peroxyl radicals have been reported to be apocarotenals and epoxy compounds (Kennedy and Liebler, 1991; Handelman et al., 1991; Mordi et al., 1993; Yamauchi et al., 1993). The 4-methoxy and 4,4′-dimethoxy derivatives have also been isolated from the reaction between β-carotene and peroxyl radicals in the presence of methanol (Woodall et al., 1997a).

Some reports suggest that the most effective carotenoids in preventing lipid peroxidation are the diketo carotenoids, canthaxanthin and astaxanthin (Terao, 1989; Miki, 1991; Palozza and Krinsky, 1992; Palozza et al., 1996). Canthaxanthin and astaxanthin have substituent groups at C-4 and C-4′, and the oxo groups in the carotenoid molecules may affect the reactivity with peroxyl radicals (Terao, 1989; Woodall et al., 1997a). Zürcher et al. (1997) have isolated 11,15′-dihydrooxepinocanthaxanthin as a major product from the oxidation of canthaxanthin with *m*-chloroperbenzoic acid. However, the reaction products of these diketo carotenoids with peroxyl radicals are still unclear.

In this study, the reaction products of canthaxanthin with peroxyl radicals generated by thermolysis of 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) in benzene were isolated and characterized. In addition, the formation of the reaction products during the AMVN-mediated peroxidation of methyl linoleate in bulk phase is described.

MATERIALS AND METHODS

Materials. *all-trans*-Canthaxanthin was obtained from Extrasynthese (Genay, France) and purified by silica gel column chromatography using benzene/ethyl acetate as the solvent system (Rosenberger et al., 1982). AMVN was obtained from Wako Pure Chemical Industries (Osaka, Japan). Methyl linoleate (Tokyo Chemical Co., Tokyo, Japan) was purified to be peroxide-free (Yamauchi et al., 1992). All other chemicals were of reagent grade. All experimental procedures were done under reduced light.

Apparatus. High-performance liquid chromatography (HPLC) was carried out with a Waters model 510 pump connected to a model 486 UV/VIS detector (Waters, Milford, MA). Ultraviolet−visible (UV−vis) spectra were measured with a Jasco Ubest-30 spectrophotometer (Japan Spectroscopic

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Co., Tokyo, Japan). Positive ion electron impact ionization mass spectrometry (EI-MS) was done with a Shimadzu 9020DF instrument (Shimadzu Co., Kyoto, Japan). Samples were introduced by a direct probe insertion and ionized with a 70-eV electron beam. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Varian Gemini 2000 instrument (Varian, Palo Alto, CA) with $CDCl_3$ as the solvent and tetramethylsilane as the internal standard. ^{1}H NMR was performed at 199.98 MHz, and the ^{1}H−^{1}H chemical shift-correlated (COSY) technique was employed to assign ^{1}H shifts and couplings.

Reaction of Canthaxanthin with AMVN. Canthaxanthin (565 mg, 1.0 mmol) and AMVN (2.48 g, 10 mmol) were dissolved in benzene (500 mL). The reaction mixture was shielded from light and incubated at 37 °C for 3 h under air. After the solvent was evaporated to dryness in vacuo, the residue was dissolved in 50 mL of ethanol and allowed to stand at −20 °C overnight. The resulting crystals of AMVN and canthaxanthin were removed by filtration. The products in the filtrate were separated by preparative HPLC. Reversed-phase HPLC was done with an Inertsil Prep-ODS column (1.0 × 25 cm; GL Sciences, Tokyo, Japan) developed with acetonitrile/water (95:5 v/v) at a flow rate of 7.0 mL/min. Normal-phase HPLC was done with a Wakosil 5Sil column (1.0 × 30 cm; Wako Pure Chemical Industries) developed with hexane/2-propanol (100:4 v/v) at 6.0 mL/min. The eluate was monitored by an absorbance at 340 nm.

Quantification of Product Distribution. Canthaxanthin (2 mM) and AMVN (20 mM) in benzene (50 mL) were incubated as described above. Periodically, an aliquot of the sample solution was taken, and benzene was removed in vacuo. The residue was dissolved in ethanol and injected into HPLC. HPLC was done with a Puresil C18 column (4.6 × 150 mm; Waters) with acetonitrile/water (95:5 v/v) at 1.0 mL/min, and the detector was set at 340 nm. The amounts of canthaxanthin and its oxidation products were determined by their peak areas calibrated against known amounts of the authentic standard.

Peroxidation of Methyl Linoleate. Methyl linoleate (1.0 g) containing canthaxanthin (0.05 mol %, based on methyl linoleate) and AMVN (2 mol %, based on methyl linoleate) was placed into a glass vial (2.4 cm in diameter) and incubated at 37 °C in the dark under air (Yamauchi et al., 1998). An aliquot of the sample was periodically withdrawn, dissolved in ethanol, and injected into the HPLC apparatus. The amount of methyl linoleate hydroperoxides (MeL-OOH) was determined by HPLC with ultraviolet detection (Yamauchi et al., 1992). Canthaxanthin was quantified by reversed-phase HPLC using a Nova-Pak C_{18} column (3.9 × 150 mm; Waters) developed with methanol at 1.0 mL/min, and the detector was set at 450 nm. To analyze the oxidation products of canthaxanthin, methyl linoleate was removed by solid-phase extraction before the HPLC analysis. The sample was dissolved in 0.5 mL of benzene and the solution applied to a silica gel column (0.5 × 3 cm). Methyl linoleate was eluted with 4 mL of benzene; then canthaxanthin and its oxidation products were eluted with 4 mL of ethyl acetate. The ethyl acetate fractions were pooled and evaporated under nitrogen gas. The residue was dissolved in ethanol, and the oxidation products of canthaxanthin were analyzed by HPLC as described above.

RESULTS

Reaction of Canthaxanthin with AMVN in Benzene. AMVN decomposes at 37 °C to form alkyl radicals which, in turn, react with molecular oxygen to form alkylperoxyl radicals. The resulting alkylperoxyl radicals can attack canthaxanthin (Yamauchi et al., 1993). The reaction products of canthaxanthin were analyzed by reversed-phase HPLC (Figure 1). Five major peaks, 1, 2, 3, 4a, and 4b, that appeared to be the reaction products of canthaxanthin, besides several minor peaks and a peak corresponding to canthaxanthin, were detected on the chromatogram. The reaction

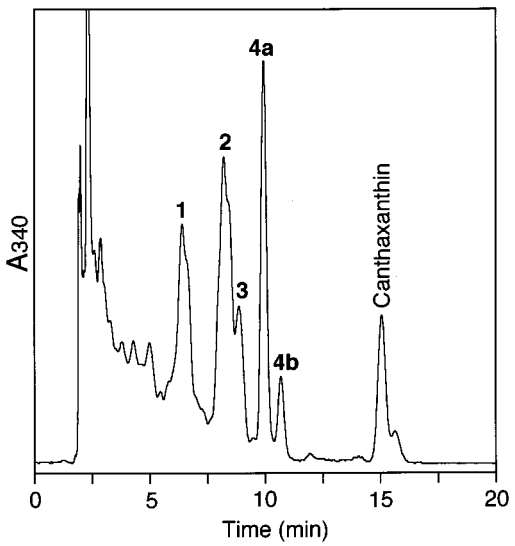


Figure 1. HPLC of an incubation mixture of canthaxanthin and AMVN in benzene at 37 °C for 3 h. HPLC was done with a Puresil C18 column (4.6 × 150 mm) developed with acetonitrile/water (95:5 v/v) at 1.0 mL/min. The eluate was monitored by an absorbance at 340 nm.

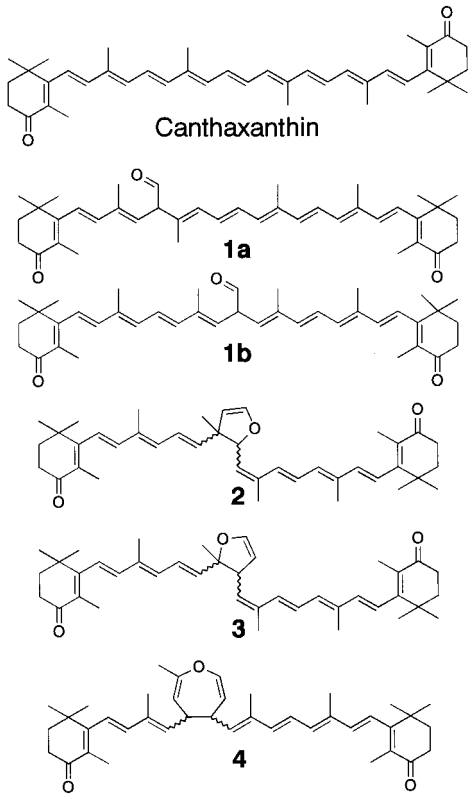


Figure 2. Structures of canthaxanthin and its oxidation products, **1**−**4**.

products, **1**−**4**, were collected by preparative reversed-phase HPLC, and their structures were characterized as follows (Figure 2).

Compound **1** was obtained as an orange solid (18.1-mg yield). The structure was shown to be a mixture of 12-formyl-11-nor-β,β-carotene-4,4′-dione (**1a**) and 15′-formyl-15-nor-β,β-carotene-4,4′-dione (**1b**) by the following spectral data: UV−vis (ethanol) λ_{max} 258 (ϵ 16 100), 330 (sh, 20 400), 370 (22 200), and 400 nm (sh, 19 400); EI-MS (70 eV) *m*/*z* 580 ($M^{+\cdot}$, 41%), 564 (13), 562 (12), 394 (23), 203 (62), 157 (49), 147 (62), 145 (60), 133 (89), 119 (83), 105 (94), 95 (100), 91 (97), and 69 (96); ^{1}H NMR

($CDCl_3$) δ 1.18 (s, 12H, H-16,17,16′17′), 1.78−1.91 (m, 4H, H-2,2′), 1.78 and 1.82 (s, 3H), 1.86 (s, 6H), 1.89 and 1.91 (s, 3H), 1.99 (s, 3H), 2.51 (m, 4H, H-3,3′), 4.03 (m, 1/4H, **1a** H-12), 4.36 and 4.47 (m, 3/4H, **1b** H-15′), 5.42 and 5.57 (m, 6/4H, **1b** H-14,14′), 5.83 (m, 1/4H, **1a** H-10), 6.16−6.68 (m, 41/4H, **1a** H-7,8,14,15,7′,8′,10′,11′,12′,14′, 15′, **1b** H-7,8,10,11,12,7′,8′,10′,11′,12′), 9.41 and 9.43 (d, J = 2.2 Hz, 3/4H, **1b** C*H*O), and 9.52 (d, J = 2.2 Hz, 1/4H, **1a** C*H*O). The ratio of compounds **1a** and **1b** was ca. 1:3 from the proton signal intensities. Peak assignments were confirmed by ^{1}H−^{1}H COSY analysis (data not shown). The spectrum contained several cross-peaks: cross-peaks corresponding to **1a** appeared between resonances at δ 4.03 (H-12) and 5.83 (H-10) and at δ 4.03 (H-12) and 9.52 (C*H*O), respectively; cross-peaks corresponding to **1b** appeared between resonances at δ 4.36 (H-15′) and 5.57 (H-14,14′), at δ 4.47 (H-15′) and 5.42 (H-14,14′), at δ 4.36 (H-15′) and 9.43 (C*H*O), and at δ 4.47 (H-15′) and 9.41 (C*H*O), respectively. Compound **1b** contained at least two geometrical isomers from the NMR spectrum, although the *E* or *Z* geometry in the conjugated polyene chain was unknown.

Compound **2** was further resolved into three peaks (**2a**−**c**) by normal-phase HPLC and obtained as yellow solids (**2a**, 10.9-mg yield; **2b**, 31.8-mg yield; **2c**, 29.5-mg yield). Compounds **2a**−**c** had similar spectral data and were identified as stereoisomers of 15′,13-epoxyvinyleno-13,15′-dihydro-14,15-dinor-β,β-carotene-4,4′-dione. **2a**: UV−vis (ethanol) λ_{max} 230 (ϵ 14 600), 279 (18 900), and 379 nm (34 900); EI-MS (70 eV) *m*/*z* 580 ($M^{+\cdot}$, 70%), 565 (7), 524 (8), 472 (8), 429 (6), 406 (13), 363 (13), 335 (10), 307 (7), 269 (49), 203 (65), 157 (39), 133 (80), 119 (76), 105 (71), 91 (65), and 69 (100); ^{1}H NMR ($CDCl_3$) δ 1.18 and 1.19 (s, 12H, H-16,17,16′,17′), 1.31 (s, 3H, deshielded H-20), 1.80−1.92 (m, 4H, H-2,2′), 1.85 (s, 6H), 1.90 (s, 3H), 1.94 (s, 3H), 1.99 (s, 3H), 2.51 (m, 4H, H-3,3′), 4.91 (d, J = 2.8 Hz, 1H, H-14), 4.94 (d, J = 8.8 Hz, 1H, H-15′), 5.53 (d, J = 9.2 Hz, 1H, H-14′), 5.60, (d, J = 15.0 Hz, 1H, H-12), 6.13−6.89 (m, 8H, H-7,8, 10,7′,8′,10′,11′,12′), 6.37 (m, 1H, H-11), 6.44 (d, J = 2.8 Hz, 1H, H-15). The ^{1}H−^{1}H COSY spectrum gave cross-peaks between resonances at δ 4.91 (H-14) and 6.44 (H-15), at δ 4.94 (H-15′) and 5.53 (H-14′), and at δ 5.60 (H-12) and 6.37 (H-11), respectively (data not shown). Since the stereochemistry of the 13- and 15′-carbon atoms was *R*,*S* or *S*,*R*, **2a** had a deshielded 20-methyl group (δ 1.31). **2b**: UV−vis (ethanol) λ_{max} 257 (ϵ 26 300), 278 (26 600), and 350 nm (51 600); EI-MS (70 eV) *m*/*z* 580 ($M^{+\cdot}$, 88%), 565 (7), 562 (7), 551 (8), 472 (6), 429 (7), 406 (17), 363 (24), 335 (12), 269 (58), 203 (67), 133 (92), 119 (87), 105 (76), 91 (76), and 69 (100); ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 3H, shielded H-20), 1.18 and 1.19 (s, 12H, H-16,17,16′,17′), 1.82−1.89 (m, 4H, H-2,2′), 1.86 (s, 9H, H-18,18′,20′), 1.95 and 1.99 (s, 6H, H-19, 19′), 2.51 (m, 4H, H-3,3′), 4.92 (d, J = 2.8 Hz, 1H, H-14), 5.01 (d, J = 9.2 Hz, 1H, H-15′), 5.69 (d, J = 8.6 Hz, 1H, H-14′), 5.89 (d, J = 9.2 Hz, 1H, H-12), 6.12−6.38 (m, 7H, H-7,8,10,7′,8′,10′,12′), 6.40 (d, J = 2.8 Hz, 1H, H-15), 6.46 (dd, J = 11.2, 15.0, 1H, H-11), and 6.62 (dd, J = 10.9, 14.9 Hz, 1H, H-11′). The ^{1}H−^{1}H COSY spectrum gave cross-peaks between resonances at δ 4.92 (H-14) and 6.40 (H-15), at δ 5.01 (H-15′) and 5.69 (H-14′), and at δ 5.89 (H-12) and 6.46 (H-11), respectively (data not shown). Since the stereochemistry of the 13- and 15′-carbon atoms was *R*,*R* or *S*,*S*, **2b** had a shielded 20-methyl group (δ 1.11). **2c**: UV−vis (ethanol) λ_{max} 257 (ϵ 23 500), 279 (26 000), and 340 nm (47 900); EI-MS (70 eV) *m*/*z* 580 ($M^{+\cdot}$, 93%), 565 (8), 562 (8), 551 (9), 472 (8), 429 (9), 406 (17), 363 (26), 335 (13), 297 (17), 269 (66), 255 (24), 203 (71), 133 (96), 119 (83), 105 (77), 91 (76), and 69 (100); ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 12H, H-16,17,16′,17′), 1.32 (s, 3H, deshielded H-20), 1.80−1.88 (m, 4H, H-2,2′), 1.85 (s, 6H, H-18,18′), 1.93 (s, 6H, H-19, 20′), 1.99 (s, 3H, H-19), 2.51 (m, 4H, H-3,3′), 4.92 (d, J = 2.6 Hz, 1H, H-14), 4.95 (d, J = 9.2 Hz, 1H, H-15′), 5.55 (d, J = 9.3 Hz, 1H, H-14′), 5.68 (d, J = 15.0 Hz, 1H, H-12), 6.11−6.46 (m, 7H, H-7,8,10,7′,8′,10′,12′), 6.41 (m, 1H, H-11), 6.45 (d, J = 2.9 Hz, 1H, H-15), and 6.63 (dd, J = 10.8, 15.0 Hz, 1H, H-11′). The ^{1}H−^{1}H COSY spectrum gave cross-peaks between resonances at δ 4.92 (H-14) and 6.45 (H-15), at δ 4.95 (H-15′) and 5.55 (H-14′), and at δ 5.68 (H-12) and 6.41 (H-11), respectively (data not shown). Since the stereochemistry of the 13- and 15′-carbon atoms was *R*,*S* or *S*,*R*, **2c** had a deshielded 20-methyl group (δ 1.32). These NMR data suggested that compound **2a** was one of the 13*R*,15′*S*- and 13*S*,15′*R*-enantiomers and **2c** was the other one and that compound **2b** was a mixture of the 13*R*,15′*R*- and 13*S*,15′*S*-enantiomers.

Compound **3** was obtained as a yellow solid (17.3-mg yield). Compound **3** had the following spectral data: UV−vis (ethanol) λ_{max} 255 nm (ϵ 21 700), 279 (20 400), and 345 nm (34 700); EI-MS (70 eV) *m*/*z* 580 ($M^{+\cdot}$, 76%), 565 (7), 562 (7), 551 (8), 537 (10), 472 (5), 429 (7), 406 (10), 363 (17), 335 (10), 269 (38), 203 (62), 133 (90), 119 (78), 105 (71), 91 (73), and 69 (100); ^{1}H NMR ($CDCl_3$) δ 1.18 and 1.19 (s, 6H, H-16,16′), 1.26 (s, 6H, H-17,17′), 1.31 and 1.49 (s, 3H, shielded and deshielded H-20), 1.82−1.88 (m, 4H, H-2,2′), 1.85 (s, 6H, H-18,18′), 1.86 (s, 3H, H-20′), 1.96 and 1.99 (s, 6H, H-19,19′), 2.51 (m, 4H, H-3,3′), 3.69 (dt, J = 2.0, 10.8 Hz, 1/2H, H-15′) and 3.77 (dt, J = 2.1, 10.3 Hz, 1/2H, H-15′), 4.77 (m, 1H, H-15), 5.33 (d, J = 10.0 Hz, 1/2H, H-14′) and 5.49 (d, J = 11.0 Hz, 1/2H, H-14′), 5.71 (d, J = 15.2 Hz, 1/2H, H-12) and 5.94 (d, J = 15.0 Hz, 1/2H, H-12), 6.17−6.47 (m, 7H, H-7,8,10,7′,8′,10′,12′), 6.39 (m, 1H, H-14), and 6.50−6.83 (m, 2H, H-11,11′). The ^{1}H−^{1}H COSY spectrum gave cross-peaks between resonances at δ 3.69 or 3.77 (H-15′) and 4.77 (H-15), at δ 3.69 (H-15′) and 5.33 (H-14′), and at δ 3.77 (H-15′) and 5.49 (H-14′), respectively (data not shown). Thus, compound **3** was identified as 13,15′-epoxyvinyleno-13,15′-dihydro-14,15-dinor-β,β-carotene-4,4′-dione. Compound **3** had two chiral carbon atoms at the 13- and 15′-positions, but the stereochemistry could not be resolved.

Compounds **4a** and **4b** were obtained as yellow solids (**4a**, 42.0-mg yield; **4b**, 23.0-mg yield). Compounds **4a** and **4b** had similar spectral data and were identified as stereoisomers of 11,15′-dihydrooxepino-β,β-carotene-4,4′-dione which has recently been reported by Zürcher et al. (1997). **4a**: UV−vis (ethanol) λ_{max} 233 (ϵ 19 500), 279 (23 700), and 340 nm (33 600); EI-MS (70 eV) *m*/*z* 580 ($M^{+\cdot}$, 82%), 565 (8), 537 (18), 524 (7), 363 (15), 269 (25), 203 (69), 133 (84), 119 (78), 105 (75), 91 (81), and 69 (100); ^{1}H NMR ($CDCl_3$) δ 1.16 and 1.19 (s, 3H), 1.26 (s, 9H), 1.81−1.88 (m, 4H, H-2,2′), 1.82 (s, 6H), 1.84 (s, 3H), 1.86 (s, 3H), 1.96 (s, 3H), 2.51 (m, 4H, H-3,3′), 3.31 (m, 2H, H-11,15′), 4.66 (dd, J = 5.7, 7.5 Hz, 1H, H-15), 4.73 (d, J = 5.9 Hz, 1H, H-12), 5.44 (d, J = 9.2 Hz, 1H, H-14′), 5.51 (d, J = 9.2 Hz, 1H, H-10), 6.03−6.40 (m, 5H, H-7,8,7′,8′,10′), 6.22 (d, J = 6.6 Hz, 1H, H-14), 6.33 (d, J = 14.1 Hz, 1H, H-12′), and 6.51 (dd, J = 10.6, 14.8 Hz, 1H, H-11′). The ^{1}H−^{1}H COSY spectrum gave cross-peaks between resonances at δ 3.31 (H-15′) and 4.66 (H-

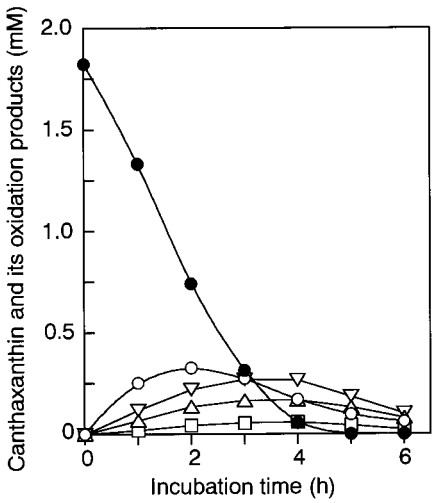


Figure 3. Kinetics of AMVN-dependent reaction of canthaxanthin and formation of compounds **1**−**4**. A reaction mixture containing 2 mM canthaxanthin and 20 mM AMVN in benzene was incubated at 37 °C under air. Symbols are (●) canthaxanthin and its reaction products: (○) compound **1**, (△) compound **2**, (□) compound **3**, and (▽) compound **4**.

15), at δ 3.31 (H-11) and 4.73 (H-12), at δ 3.31 (H-15′) and 5.44 (H-14′), at δ 3.31 (H-11) and 5.51 (H-10), and at δ 4.66 (H-15) and 6.22 (H-14), respectively (data not shown). **4b**: UV−vis (ethanol) λ_{max} 232 (ϵ 20 300), 280 (24 800), and 333 nm (29 000); EI-MS (70 eV) *m*/*z* 580 ($M^{+\cdot}$, 67%), 565 (6), 537 (13), 524 (6), 363 (12), 269 (21), 203 (54), 133 (72), 119 (66), 105 (64), 91 (67), and 69 (100); ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 3H), 1.19 (s, 6H), 1.26 (s, 3H), 1.80−1.87 (m, 4H, H-2,2′), 1.82 (s, 6H), 1.84 (s, 3H), 1.86 (s, 3H), 1.89 and 1.98 (s, 3H), 2.50 (m, 4H, H-3,3′), 3.29 (m, 1H, H-11), 3.42 (m, 1H, H-15′), 4.65 (m, 1H, H-15), 4.74 (d, J = 6.4 Hz, 1H, H-12), 5.31 (d, J = 9.9 Hz, 1H, H-14′), 5.52 (d, J = 9.0 Hz, 1H, H-10), 6.04−6.89 (m, 8H, H-7,8,10,7′,8′,10′,11′,12′), 6.23 (d, J = 13.6 Hz, 1H, H-14). The ^{1}H−^{1}H COSY spectrum (not shown) gave cross-peaks between resonances at δ 3.29 (H-11) and 4.74 (H-12), at δ 3.29 (H-11) and 5.52 (H-10), at δ 3.42 (H-15′) and 4.65 (H-15), at δ 3.42 (H-15) and 5.31 (H-14′), and at δ 4.65 (H-15) and 6.23 (H-14), respectively. Compounds **4a** and **4b** had two chiral carbon atoms at the 11- and 15′-positions, although stereochemistry of the 11- and 15′-positions could not be resolved from the present spectral data.

Aliquots of the reaction mixture were taken during the reaction and analyzed by reversed-phase HPLC to determine the distribution of products and the relative rates of formation of each product (Figure 3). The oxidation products of canthaxanthin, **1**−**4**, accumulated with decrease of canthaxanthin at the initial stage. Thereafter, compound **1** gradually disappeared, but compounds **2**−**4** were somewhat stable.

Effect of Canthaxanthin on the AMVN-Initiated Peroxidation of Methyl Linoleate. Methyl linoleate containing canthaxanthin (0.05 mol %) was oxidized at 37 °C by 2 mol % AMVN in bulk phase. The reaction mixture contains a larger amount of methyl linoleate than canthaxanthin. Therefore, most of the alkylperoxyl radicals generated by thermolysis of AMVN attack methyl linoleate to generate the pentadienyl radical, which reacts with oxygen to give the methyl linoleate-peroxyl radical (Yamauchi et al., 1990). Then, canthaxanthin can react with the methyl linoleate-peroxyl radical. Figure 4 shows the formation of MeL-OOH, the

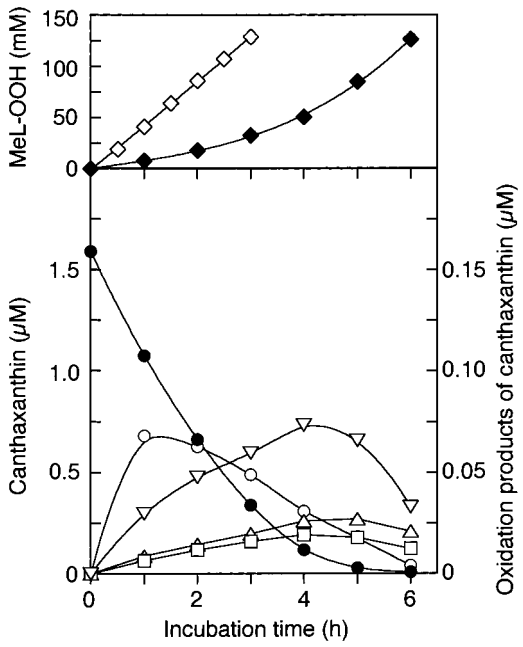


Figure 4. Effect of canthaxanthin on the AMVN-initiated peroxidation of methyl linoleate. Methyl linoleate containing 0.05 mol % canthaxanthin was oxidized at 37 °C under air by the addition of 2 mol % AMVN. Symbols are MeL-OOH (◇) without or (◆) with canthaxanthin, (●) canthaxanthin, and the reaction products of canthaxanthin: (○) compound **1**, (△) compound **2**, (□) compound **3**, and (▽) compound **4**.

Table 1. Product Distributions from Canthaxanthin[a]

reaction system	reaction time, h	yield (%)[b] canthaxanthin	1	2	3	4
in benzene	1	73.1	13.8	3.7	1.2	6.5
	2	40.7	17.9	7.5	2.4	12.1
	4	3.1	9.4	9.2	3.3	14.6
in methyl linoleate	1	67.7	4.3	0.6	0.4	1.9
	2	41.8	4.0	0.9	0.8	3.0
	4	7.5	1.9	1.6	1.2	4.6

[a] These data are from the experiments described in Figures 3 and 4. [b] Mol % to each theoretical yield based on the starting material.

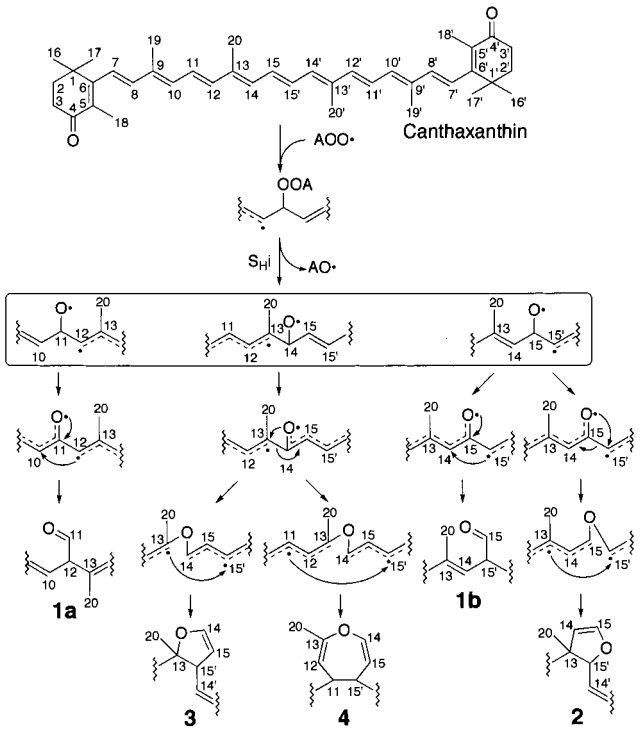
fate of canthaxanthin, and the formation of canthaxanthin products. The peroxidation of methyl linoleate was suppressed by 0.05 mol % canthaxanthin, although it did not produce a clear induction period or lag phase. The oxidation product of canthaxanthin first formed was compound **1**, after which compounds **2**−**4** accumulated in the reaction mixture.

Table 1 compares the relative product yields of canthaxanthin during the AMVN-initiated reaction in benzene and in methyl linoleate. Compounds **1**−**4** were relatively stable and accounted for 94% of the consumed canthaxanthin in the 1-h reaction mixture in benzene. On the other hand, compounds **1**−**4** accounted for only 22% of the consumed canthaxanthin in the 1-h reaction mixture during the AMVN-initiated peroxidation of methyl linoleate.

DISCUSSION

Carotenoids are excellent substances for free radical attack because they have long and conjugated double bonds in the molecule. The peroxyl radical addition reaction of β-carotene has resulted in the formation of several oxygenated compounds, including carbonyl compounds and epoxides as well as chain-cleavage products

Scheme 1. Proposed Reaction Pathway for the Peroxyl Radical Reaction of Canthaxanthin



and polymeric products (El-Tinay and Chichester, 1970; Kennedy and Liebler, 1991; Handelman et al., 1991; Yamauchi et al., 1993; Mordi et al., 1993; Tsuchihashi et al., 1995). In this study, we have isolated and characterized the reaction products of canthaxanthin with the alkylperoxyl radicals from AMVN. The products are a mixture of 12-formyl-11-nor-β,β-carotene-4,4′-dione (**1a**) and 15′-formyl-15-nor-β,β-carotene-4,4′-dione (**1b**), 15′,13-epoxyvinyleno-13,15′-dihydro-14,15-dinor-β,β-carotene-4,4′-dione (**2**), 13,15′-epoxyvinyleno-13,15′-dihydro-14,15-dinor-β,β-carotene-4,4′-dione (**3**), and 11,-15′-dihydrooxepino-β,β-carotene-4,4′-dione (**4**) (Figure 2). The formation of formyl (**1**) and dihydrofuran (**2** and **3**) derivatives has already been observed in our previous work (Yamauchi et al., 1993, 1998). The structure of dihydrooxepin derivative (**4**) was recently assigned to a compound isolated as a major product of the oxidation of canthaxanthin with *m*-chloroperbenzoic acid (Zürcher et al., 1997). The dihydrofuran and dihydrooxepin derivatives have been observed as the thermal rearrangement of diethylenic epoxides (Pommelet et al., 1972). Therefore, it is likely that compound **2** may be a rearrangement product of canthaxanthin 15,15′-epoxide and compounds **3** and **4** being those of the 13,14-epoxide, respectively.

Carotenoids have been proposed to exert antioxidant activities by a mechanism in which the chain-propagating peroxyl radical is trapped by addition to the conjugated polyene system (Burton and Ingold, 1984). Scheme 1 shows a possible reaction pathway for the reaction of canthaxanthin with AMVN-derived peroxyl radicals in benzene. The first step is peroxyl radical (AOO·) addition to the polyene chain of canthaxanthin. This direct addition takes place to form a resonance-stabilized, carbon-centered radical, which then decomposes by intramolecular homolytic substitution (S_Hi) to yield an epoxide and an alkoxyl radical, reacts with a second peroxyl radical to form a nonradical addition product, or reacts with oxygen to form a carotenoid-peroxyl radical (Kennedy and Liebler, 1992; Mordi et al., 1993; Tsuchihashi et al., 1995; Liebler and McClure, 1996). In the present study, we obtained compounds **1**−**4** as the oxidation products of canthaxanthin, which accounted for almost all the consumed canthaxanthin (Figure 3 and Table 1). The result indicates that the peroxycarbon-centered radicals of canthaxanthin might undergo S_Hi reaction, yielding alkoxyl radicals (AO·) from the added peroxyl radical and alkoxyl radicals of canthaxanthin. The alkoxyl radicals of canthaxanthin then rearrange into oxo-allylic radicals to form carbonyl compounds (**1a** and **1b**) or rearrange into epoxy-allylic radicals to form epoxy compounds (**2**−**4**). Since compound **2** was obtained as the all-possible optical isomers, **2a**−**c**, with different yields, the rearrangement of canthaxanthin-alkoxyl radicals might proceed stereoselectively. However, the stereochemical reaction mechanism of canthaxanthin is still unclear. Furthermore, the product distribution indicates that the peroxyl radical adds at the central parts of the polyene structure of canthaxanthin molecule (Scheme 1 and Table 1). Alternatively, there may be other routes by which some of the peroxycarbon-centered radicals are reacted with a second peroxyl radical or atmospheric oxygen (Kennedy and Liebler, 1992; Tsuchihashi et al., 1995; Liebler and McClure, 1996). In our reaction system, the generation rate of peroxyl radicals was high and the concentration of oxygen was low relative to the concentration of peroxyl radicals formed. Therefore, the reaction between the peroxycarbon-centered radicals and a second peroxyl radical might be favored to form nonradical products (Liebler and McClure, 1996). However, we could not detect such products under our experimental conditions. The hydrogen abstraction from allylic C-4 and C-4′ positions of β-carotene has also been postulated as one of the possible mechanisms that occur when β-carotene is exposed to peroxyl radicals, along with the peroxyl addition and electron capture (Woodall et al., 1997a). In contrast, the possibility of the hydrogen abstraction is removed in canthaxanthin that has oxo groups at the C-4 and C-4′ positions.

Canthaxanthin has been reported to be a more effective antioxidant against peroxyl radical-initiated peroxidation when compared with β-carotene (Terao, 1989; Palozza and Krinsky, 1992). In the present study, canthaxanthin could reduce the AMVN-derived peroxyl radical-mediated formation of MeL-OOH from methyl linoleate in bulk phase (Figure 4). In this antioxidant process, canthaxanthin was consumed and compounds **1**−**4** were detected as the reaction products with low yield (Table 1). This result indicates that the peroxyl radical addition occurred in the antioxidant process of canthaxanthin during the AMVN-initiated peroxidation of methyl linoleate. It has been proposed that substitution of the hydrogen atoms with carbonyl groups at the C-4 and C-4′ positions enhances the stability of trapped radical by decreasing its tendency for continued chain-propagating reaction (Mortensen et al., 1997). Thus, the keto carotenoids are more effective antioxidants against lipid peroxidation (Terao, 1989; Miki, 1991; Palozza and Krinsky, 1992; Palozza et al., 1996). Woodall et al. (1997b) have demonstrated that the antioxidant ability of carotenoids is positively correlated with carotenoid radical reactivity with the exception of lycopene when the systems are subjected to attack by a high concentration of free radicals over a short time; the reactivity and protective ability of canthaxanthin were less than those

of β-carotene. When the peroxidation was carried out with low rates of radical production, carotenoids such as canthaxanthin and astaxanthin, which are less reactive toward free radicals, would less readily autoxidize compared with β-carotene and would be present for longer periods to intercept free radicals. Thus, canthaxanthin might be an effective antioxidant in such conditions.

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