Nickel Peroxide Induced Oxidation of Canthaxanthin

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Treatment of canthaxanthin (*β*,*β*-carotene-4,4′-dione) (**1**) with nickel peroxide in dichloromethane yielded a series of cleavage products, i.e., 4-oxo-*β*-ionone (**2**), (7*E*,9*E*)-4-oxo-*β*-apo-11-carotenal (**3a**), (7*E*,9*Z*)-4-oxo-*β*-apo-11-carotenal (**3b**), 4-oxo-*β*-apo-13-carotenone (**4**), 4-oxo-*β*-apo-14′-carotenal (**5**), 4-oxo-*β*-apo-12′-carotenal (**6**), and 4-oxo-*β*-apo-10′-carotenal (**7**). In addition, oxidized canthaxanthin derivatives, i.e., isomeric ketols all-*trans*-9,10-dihydro-9-hydroxy-10-oxo-canthaxanthin (**8a**), (9′*Z*)-9,10-dihydro-9-hydroxy-10-oxo-canthaxanthin (**8b**), and (13′*Z*)-9,10-dihydro-9-hydroxy-10-oxo-canthaxanthin (**8c**) were obtained together with the tentatively identified (9′*Z*)-canthaxanthin-20-al (**9**). Structure elucidation of the reaction products was achieved by mass spectrometry and two-dimensional NMR spectroscopy.

Keywords: *Canthaxanthin; oxidation; degradation; apocarotenoids; nickel peroxide*

INTRODUCTION

Whereas the oxidative degradation of *β*-carotene has been thoroughly studied under various conditions (Handelman et al., 1991; Stratton et al., 1993; Yamauchi et al., 1993), little is known about oxidative conversions of canthaxanthin (**1**). Cyronak et al. (1978) reported the isolation of two degradation products, i.e., 4-oxo-*β*-apo-14′-carotenal and 4-oxo-*β*-apo-12′-carotenal, after column chromatography of canthaxanthin monoepoxy derivatives on adsorptive magnesia. Zürcher et al. (1997) obtained a dihydrooxepin derivative after the oxidation of canthaxanthin with *m*-chloroperbenzoic acid. El-Tinay and Chichester (1970) together with Mordi et al. (1993) discussed the likely formation of 4-oxo derivatives in the course of the oxidation of *β*-carotene. The generation of these derivatives was explained by the resonance stability of the allylic carbon in positions 4/4′ of the *β*-carotene chain being stabilized over 11 double bonds. Moreover, like in the case of other carotenoids, the antioxidative potential of canthaxanthin (**1**) has attracted attention. Studies with different kinds of free radicals (Jorgensen and Skibsted, 1993; Hill et al., 1995) demonstrated a protective function of canthaxanthin against radical destruction of cells. In animal tumor studies (Mathews-Roth and Krinsky, 1985), a possible role of canthaxanthin (**1**) in the prevention of UV-light-induced skin cancer was apparent. Since the metabolic pathways of canthaxanthin are largely unknown, this study provides some spectral data for canthaxanthin metabolites that will facilitate identification of these compounds in natural tissues.

MATERIALS AND METHODS

Materials. Canthaxanthin (*β*,*β*-carotene-4,4′-dione) was donated by Roche Vitamine Division (Basel, Switzerland) and was used without further purification. Nickel peroxide was prepared according to the method of Nakagawa et al. (1962). All commercial chemicals used were of analytical grade quality. Solvents were redistilled before use. All experiments were carried out in the dark.

Instrumentation. High-performance liquid chromatography (HPLC) was performed with a Jasco model PU-980 HPLC pump (Jasco, Gross-Umstadt, Germany), a Jasco DG-988-50 degasser, and a Jasco LG-980-02 ternary gradient unit. The eluent was monitored by a Jasco MD-910 multiwavelength detector. UV spectra were measured on-line with the Jasco MD-910 multiwavelength detector. High-resolution gas chromatography−mass spectrometry (HRGC−MS) was performed with a Hewlett-Packard GCD system equipped with a PTV injector (KAS-system, Gerstel, Mülheim, Germany). For HRGC−MS, a J&W fused silica DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 *μ*m) was used. The temperature program was from 60 °C (2 min isothermal) to 300 °C at 5 °C/min. Carrier gas flow rate 1.2 mL/min He; temperature of ion source, 180 °C; electron energy, 70 eV; injection volumes, 1 *μ*L. The linear retention index (R_i) is based on a series of *n*-hydrocarbons. ^{1}H and ^{13}C NMR spectral data were recorded on Fourier transform Bruker AM 360 and AC 250 spectrometers with TMS as internal reference standard. Signals were assigned by ^{1}H−^{13}C COSY.

Oxidation of Canthaxanthin (1). To a solution of canthaxanthin (1.2 g) in 1.2 L of dichloromethane, nickel peroxide (70 g) was added. After stirring the solution for 5 h under nitrogen, nickel peroxide was removed by filtration over Celite, and the reaction mixture was concentrated to a volume of 5 mL.

Isolation of Oxidation Products. The fractionation of the reaction mixture was achieved by flash chromatography (Still et al., 1978) on silica gel using a hexane/*tert*-butyl methyl ether (TBME) gradient as well as subsequent HPLC on an Eurospher Si 100 column (5 *μ*m, 250 × 4 mm, Knauer, Berlin, Germany; flow rate, 1 mL/min; eluent, hexane/TBME 95/5, 80/20, and 75/25 v/v). HPLC retention times (RT) were determined on a YMC Carotenoid C30 column (5 *μ*m, 250 × 4.6 mm, YMC Europe GmbH, Schermbeck, Germany; flow rate, 1 mL/min; eluent A, MeOH/TBME/H_2O 81/15/4; eluent B, MeOH/TBME/H_2O 6/90/4; gradient, 1−100% B in 90 min; cf. Table 1).

The following compounds have been obtained in pure form:

4-Oxo-*β*-ionone (2). Spectral data were identical with those published by Becher et al. (1981).

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Table 1. UV/Vis Maxima (λ_{Max}) and HPLC Retention Time (RT) on a YMC Carotenoid C30 Column for Compounds 2−9

compd	λ_{max} (nm)	RT (min)	compd	λ_{max} (nm)	RT (min)
9	484	8.6	**5**	408	4.0
8a	460	4.6	**4**	344	3.5
8b	452	5.2	**3a**	304	3.3
8c	448, 352	5.0	**3b**	300, 252	3.4
7	456	5.6	**2**	276	3.2
6	432	4.6			

(7*E*,9*E*)-4-Oxo-*β*-apo-11-carotenal (3a). R_i (DB-5) 2021; EI−MS *m*/*z* 232 (M^+, 9), 217 (5), 203 (14), 189 (13), 171 (5), 161 (12), 148 (15), 147 (13), 133 (25), 119 (15), 115 (9), 105 (19), 96 (11), 95 (100), 91 (24), 77 (11), 65 (5), 55 (6); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.19 (6H, s, H-12 and H-13), 1.82 (3H, d, *J* = 1 Hz, H-14), 1.89 (2H, t, *J* = 7 Hz, H-2), 2.35 (3H, d, *J* = 1 Hz, H-15), 2.54 (2H, t, *J* = 7 Hz, H-3), 6.02 (1H, dd, *J* = 8, 1 Hz, H-10), 6.32 (1H, d, *J* = 16 Hz, H-8), 6.74 (1H, d, *J* = 16 Hz, H-7), 10.17 (1H, d, *J* = 8 Hz, H-11); ^{13}C NMR (63 MHz, $CDCl_3$): *δ* 12.9 (C-15), 13.6 (C-14), 27.5 (C-12 and C-13), 34.2 (C-3), 35.7 (C-1), 37.3 (C-2), 130.7 (C-7), 131.1 (C-5), 132.5 (C-9), 139.3 (C-8), 152.8 (C-10), 159.2 (C-6), 191.1 (C-11), 198.8 (C-4).

(7*E*,9*Z*)-4-Oxo-*β*-apo-11-carotenal (3b). R_i (DB-5) 1971; EI−MS *m*/*z* 232 (M^+, 4), 203 (12), 189 (12), 171 (5), 161 (12), 148 (12), 147 (12), 133 (21), 119 (16), 117 (9), 115 (14), 105 (24), 96 (10), 95 (100), 93 (8), 91 (36), 79 (15), 77 (18), 65 (11), 55 (12), 41 (4); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.21 (6H, s, H-12 and H-13), 1.85 (3H, d, *J* = 1 Hz, H-14), 1.90 (2H, t, *J* = 7 Hz, H-2), 2.17 (3H, d, *J* = 1 Hz, H-15), 2.54 (2H, t, *J* = 7 Hz, H-3), 6.00 (1H, dd, *J* = 8, 1 Hz, H-10), 6.65 (1H, d, *J* = 16 Hz, H-7), 7.22 (1H, d, *J* = 16 Hz, H-8), 10.15 (1H, d, *J* = 8 Hz, H-11); ^{13}C NMR (63 MHz, $CDCl_3$): *δ* 13.7 (C-14), 20.9 (C-15), 27.5 (C-12 and C-13), 34.2 (C-3), 35.7 (C-1), 37.3 (C-2), 129.7 (C-7), 130.5 (C-9), 131.1 (C-5), 133.7 (C-8), 152.8 (C-10), 159.2 (C-6), 189.6 (C-11), 198.8 (C-4).

4-Oxo-*β*-apo-13-carotenone (4). R_i (DB-5) 2402; EI−MS *m*/*z* 272 (M^+, 33), 257 (57), 239 (7), 229 (32), 215 (54), 201 (52), 197 (37), 187 (41), 173 (84), 159 (63), 157 (39), 145 (54), 143 (36), 141 (33), 133 (30), 131 (33), 129 (41), 128 (50), 122 (44), 119 (42), 117 (31), 115 (71), 109 (55), 105 (50), 91 (100), 79 (35), 77 (49), 65 (26), 55 (28), 43 (47); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.19 (6H, s, H-15 and H-16), 1.84 (3H, d, *J* = 1 Hz, H-17), 1.87 (2H, t, *J* = 7 Hz, H-2), 2.10 (3H, d, *J* = 1 Hz, H-18), 2.31 (3H, s, H-14), 2.52 (2H, t, *J* = 7, H-3), 6.23 (1H, d, *J* = 15 Hz, H-12), 6.27 (1H, d, *J* = 12 Hz, H-10), 6.37 (1H, d, *J* = 16 Hz, H-8), 6.45 (1H, d, *J* = 16 Hz, H-7), 7.56 (1H, dd, *J* = 15, 12 Hz, H-11); ^{13}C NMR (63 MHz, $CDCl_3$): *δ* 13.0 (C-18), 13.7 (C-17), 27.6 (C-15 and C-16), 28.0 (C-14), 34.3 (C-3), 35.7 (C-1), 37.4 (C-2), 128.3 (C-7), 130.5/7/130.8 (C-5, C-9, and C-10), 138.2 (C-12), 139.6 (C-8), 143.7 (C-11), 160.2 (C-6), 198.2 (C-13), 199.1 (C-4).

4-Oxo-*β*-apo-14′-carotenal (5). EI−MS *m*/*z* 324 (M^+, 86), 309 (31), 255 (19), 239 (20), 225 (25), 221 (55), 215 (28), 211 (27), 207 (49), 195 (27), 183 (35), 171 (39), 157 (53), 147 (82), 133 (74), 119 (70), 115 (57), 105 (98), 91 (100), 77 (47), 69 (30), 55 (30); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.19 (6H, s, H-16 and H-17), 1.87 (3H, s, H-18), 1.87 (2H, t, *J* = 7 Hz, H-2), 2.04 (3H, s, H-20), 2.12 (3H, s, H-19), 2.51 (2H, t, *J* = 7 Hz, H-3), 6.20 (1H, dd, *J* = 15, 8 Hz, H-15′), 6.28 (1H, d, *J* = 11.5 Hz, H-10), 6.32 (1H, d, *J* = 16 Hz, H-7), 6.36 (1H, d, *J* = 16 Hz, H-8), 6.39 (1H, d, *J* = 12 Hz, H-14), 6.46 (1H, d, *J* = 15 Hz, H-12), 6.91 (1H, dd, *J* = 15, 11.5 Hz, H-11), 7.51 (1H, dd, *J* = 15, 12 Hz, H-15), 9.63 (1H, d, *J* = 8 Hz, H-14′); ^{13}C NMR (63 MHz, $CDCl_3$): *δ* 12.8 (C-19), 13.3 (C-20), 13.8 (C-18), 27.6 (C-16 and C-17), 34.3 (C-3), 35.7 (C-1), 37.4 (C-2), 125.9 (C-7), 129.0 (C-15′), 129.5 (C-11), 130.2 (C-5), 131.4/133.3/137.4/137.8 (C-9,10,12,13), 140.6 (C-8), 146.0 (C-14), 147.1 (C15), 160.8 (C-6), 193.5 (C-14′), 199.2 (C-4).

4-Oxo-*β*-apo-12′-carotenal (6). Spectral data were identical with those published by Bernhard et al. (1981).

4-Oxo-*β*-apo-10′-carotenal (7). EI−MS *m*/*z* 390 (M^+, 74), 321 (9), 279 (6), 267 (9), 237 (12), 223 (16), 209 (26), 195 (37), 187 (46), 171 (50), 157 (78), 145 (100), 133 (74), 119 (63), 105 (92), 91 (94), 79 (33), 69 (38), 55 (36); 43 (19); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.20 (6H, s, H-16 and H-17), 1.86 (2H, t, *J* = 7 Hz, H-2), 1.87 (3H, s, H-18), 1.97 (3H, s, H-20′), 2.02 (3H, s, H-19), 2.03 (3H, s, H-20), 2.51 (2H, t, *J* = 7 Hz, H-3), 6.20 (1H, dd, *J* = 15.5, 8 Hz, H-11′), 6.27 (1H, d, *J* = 16 Hz, H-7), 6.28 (1H, d, *J* = 11.5 Hz, H-10), 6.34 (1H, d, *J* = 12 Hz, H-14), 6.37 (1H, d, *J* = 16 Hz, H-8), 6.43 (1H, d, *J* = 15 Hz, H-12), 6.64 (1H, d, *J* = 12 Hz, H-14′), 6.66 (1H, dd, *J* = 12, 12 Hz, H-15), 6.74 (1H, dd, *J* = 15, 11.5 Hz, H-11), 6.87 (1H, m, H-15′), 7.16 (1H, d, *J* = 15.5 Hz, H-12′), 9.60 (1H, d, *J* = 8 Hz, H-10′).

All-*trans*-9,10-dihydro-9-hydroxy-10-oxo-canthaxanthin (8a). EI−MS *m*/*z* 596 (M^+, 9), 208 (73), 203 (80), 159 (54), 157 (43), 147 (41), 145 (61), 137 (43), 133 (100), 121 (58), 119 (65), 109 (36), 105 (62), 95 (35), 91 (63), 69 (37), 55 (31); EI−MS (after silylation with MSTFA) *m*/*z* 668 (M^+, 2), 389 (5), 279 (100), 73 (88); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.12, 1.13, 1.19, 1.20 (12H, s, H-16/16′, H-17/17′), 1.57 (3H, s, H-19), 1.76 (3H, s, H-18), 1.84, 1.86 (4H, t, *J* = 7 Hz, H-2/2′), 1.87 (3H, s, H-18′), 1.95 (3H, s, H-20), 2.02 (3H, s, H-19′), 2.03 (3H, s, H-20), 2.49/2.51 (4H, t, *J* = 7 Hz, H3/3′), 5.75 (1H, d, *J* = 16 Hz, H-8), 6.27 (1H, d, *J* = 16 Hz, H-7′), 6.28 (1H, d, *J* = 12 Hz, H-10′), 6.32 (1H, d, *J* = 12 Hz, H-14′), 6.36 (1H, d, *J* = 16 Hz, H-8′), 6.39 (1H, d, *J* = 15 Hz, H-11), 6.43 (1H, d, *J* = 15 Hz, H-12′), 6.46 (1H, d, *J* = 16 Hz, H-7), 6.65 (2H, m, H-14/15), 6.75 (1H, dd, *J* = 15, 11.5 Hz, H-11′), 6.86 (1H, m, H-15′), 7.58 (1H, d, *J* = 15 Hz, H-12); ^{13}C NMR (63 MHz, $CDCl_3$): *δ* 12.6 (C-19′), 12.7 (C-20′),13.0 (C-20), 13.4 (C-18), 13.8 (C-18′), 25.1 (C-19), 27.0 (C-16), 27.3 (C-16′/C17), 27. 7 (C-17′), 34.3 (C-3 and 3′), 35.6, 35.7 (C-1/1′), 37.2, 37.4 (C-2/2′), 77.2 (C-9), 116.9 (C-11), 124.8 (C-7′), 126.3 (C-11′), 127.7 (C-7), 129.2 (C-15′), 129.9, 130.0 (C-5/5′), 132.7 (C-14′), 133.7 (C-9′), 134.0 (C-10′), 135.2 (C-15), 135.9 (C-13′), 137.0 (C-8), 138.7 (C-12′), 139. 9 (C-13), 141.1 (C-8′), 142.7 (C-14), 150.2 (C-12), 160.6 (C-6), 161.0 (C-6′), 198.5 (C-10), 199.2 (C-4/4′).

(9′*Z*)-9,10-Dihydro-9-hydroxy-10-oxo-canthaxanthin (8b). EI−MS *m*/*z* 596 (M^+, 4), 355 (44), 281 (65), 221 (100), 208 (33), 192 (37), 159 (31), 147 (87), 145 (36), 133 (52), 121 (38), 119 (43), 105 (42), 95 (27), 91 (55), 73 (31), 69 (30), 55 (27); EI−MS (after silylation with MSTFA) *m*/*z* 668 (M^+, 0.5), 389 (2), 279 (3), 73 (17); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.12, 1.14, 1.19, 1.20 (12H, s, H-16/16′, H-17/17′), 1.57 (3H, s, H-19, signal obscured), 1.76, (3H, s, H-18), 1.84, 1.88 (4H, t, *J* = 7 Hz, H-2/2′), 1.91 (3H, s, H-18′), 1.94 (3H, s, H-20), 2.00 (3H, s, H-19′), 2.02 (3H, s, H-20′), 2.49, 2.53 (4H, t, *J* = 7 Hz, H-3/3′), 5.75 (1H, d, *J* = 16 Hz, H-8), 6.21 (1H, d, *J* = 11.5 Hz, H-10′), 6.29 (1H, d, *J* = 16 Hz, H-7′), 6.30 (1H, d, *J* = 12 Hz, H-14′), 6.36 (1H, d, *J* = 15.5 Hz, H-12′), 6.39 (1H, d, *J* = 15 Hz, H-11), 6.46 (1H, d, *J* = 16 Hz, H-7), 6.65 (2H, m, H-14/15), 6.80 (1H, dd, *J* = 15, 11.5 Hz, H-11′), 6.86 (1H, m, H-15′), 6.89 (1H, d, *J* = 16, H-8′), 7.58 (1H, d, *J* = 15 Hz, H-12).

(13′*Z*)-9,10-Dihydro-9-hydroxy-10-oxo-canthaxanthin (8c). UV−Vis maxima at *λ* 448 and 352 nm; EI-MS *m*/*z* 596 (M^+, 12), 208 (70), 207 (40), 203 (77), 192 (55), 191 (71), 185 (51), 163 (58), 159 (62), 157 (50), 147 (52), 145 (74), 137 (63), 133 (100), 121 (80), 119 (80), 109 (56), 107 (57), 105 (83), 95 (53), 91 (88), 69 (66), 55 (60), 43 (43); EI-MS (after silylation with MSTFA) *m*/*z* 668 (M^+, 1), 389 (2), 279 (39), 73 (100); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.12, 1.13, 1.19, 1.20 (12H, s, H-16/16′, H-17/17′), 1.57 (3H, s, H-19, signal obscured), 1.76 (3H, s, H-18), 1.84, 1.86 (4H, t, *J* = 7 Hz, H-2/2′), 1.87 (3H, s, H-18′), 1.95 (3H, s, H-20), 2.02 (3H, s, H-19′), 2.04 (3H, s, H-20′), 2.49, 2.52 (4H, t, *J* = 7, H-3/3′), 5.75 (1H, d, *J* = 16 Hz, H-8), 6.18 (1H, d, *J* = 12 Hz, H-14′), 6.29 (1H, d, *J* = 16 Hz, H-7′), 6.34 (1H, d, *J* = 11,5 Hz, H-10′), 6.38 (1H, d, *J* = 16 Hz, H-8′), 6.39 (1H, d, *J* = 16 Hz, H-11), 6.46 (1H, d, *J* = 16 Hz, H-7), 6.58 (1H, m, H-15), 6.65 (1H, m, H-14), 6.74 (1H, dd, *J* = 15, 11.5 Hz, H-11′), 6.96 (1H, d, *J* = 15.5 Hz, H-12′), 7.01 (1H, dd, *J* = 15, 11.5 Hz, H-15′), 7.58 (1H, d, *J* = 15 Hz, H-12).

(9′*Z*)-Canthaxanthin-20-al (9). EI−MS *m*/*z* 578 (M^+, 33), 215 (48), 203 (93), 191 (46), 185 (46) 183 (48), 169 (45), 167 (65), 159 (42), 157 (45), 149 (100), 147 (50), 133 (87), 121 (52), 105 (61), 95 (47), 91 (59), 85 (43), 83 (45), 81 (48), 69 (97), 57 (94), 43 (51); ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 1.19, 1.19, 1.20, 1.21 (12H, s, H-16/16′, H-17/17′), 1.86 (4H, t, *J* = 7 Hz, H-2/

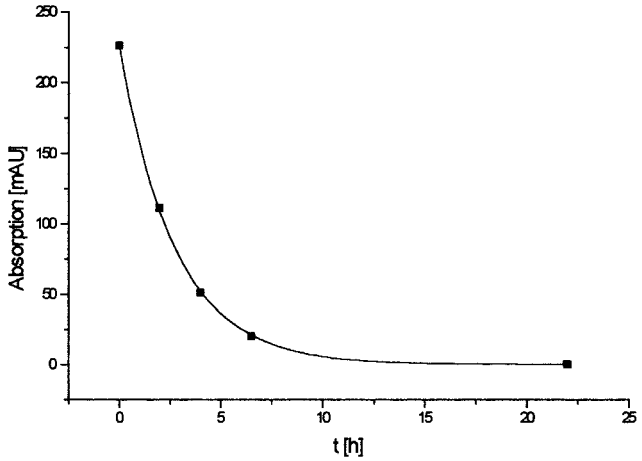

Figure 1. Kinetics of nickel peroxide induced degradation of canthaxanthin (**1**).

2′), 1.87 (3H, s, H-18′), 1.91 (3H, s, H-18), 2.03, 2.04, 2.07 (9H, s, H-19/19′, H-20′), 2.52, 2.53 (4H, t, J = 7 Hz, H-3/3′), 6.21 (1H, d, J = 11.5 Hz, H-10′), 6.30 (1H, d, J = 16 Hz, H-7′), 6.31 (1H, d, J = 12 Hz, H-14′), 6.32 (1H, d, J = 16 Hz, H-7), 6.36 (1H, d, J = 11.5 Hz, H-10), 6.38 (1H, d, J = 16 Hz, H-12′), 6.46 (1H, d, J = 16 Hz, H-8), 6.51 (1H, d, J = 15 Hz, H-12), 6.82 (1H, dd, J = 15, 11.5 Hz, H-11′), 6.84 (1H, d, J = 12 Hz, H-14), 6.89 (1H, m, H-15′), 6.96 (1H, d, J = 16 Hz, H-8′), 7.09 (1H, m, H-15), 7.82 (1H, dd, J = 15, 11.5 Hz, H-11), 9.55 (1H, d, J = 2 Hz, H-20).

Silylation of Ketols 8a−c. To 0.2 mg of each of the dry compounds, 50 μL of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was added. After standing overnight at room temperature, MSTFA was removed by a gentle stream of dry nitrogen. The silylated compounds were dissolved in dichloromethane (50 μL) for subsequent MS analysis.

RESULTS AND DISCUSSION

The use of nickel peroxide for the gentle oxidation of carotenoids has been reported by Liaaen-Jensen and Hertzberg (1966), Buchecker et al. (1974), and Hohler (1986). In the case of lutein (3,3′-dihydroxy-α-carotene), the last-mentioned author was able to identify a whole series of volatile degradation products after nickel peroxide treatment. In addition to volatiles such as 3-oxo-α-ionone, 3-hydroxy-β-ionone, and 3,4-dehydro-β-ionone, several higher molecular degradation products (apocarotenoids) were also detected whose molecular structure remained obscure. In continuation of this work, we decided to use nickel peroxide for the oxidation of the 4-oxocarotenoid canthaxanthin (**1**). Due to the apparent nonselectivity of the oxidation with regard to the site of the attack as well as the gentle oxidation conditions (room temperature), the formation of a series of 4-oxo-apocarotenals and 4-oxo-apocarotenones was considered as being likely. Such 4-oxo compounds have been tentatively identified by Mordi et al. (1993) as oxidation products of β-carotene. The lack of reference data and the tiny amount of products formed excluded so far a confirmation of the postulated structure of the cleavage products. Consequently, a preparation of authentic references through nickel peroxide treatment of canthaxanthin (**1**) was an important prerequisite to solve the identification problems.

In preliminary tests, the reaction time, the amount of nickel peroxide, and the solvent system used for the oxidation of canthaxanthin (**1**) have been optimized in a way that major amounts of apocarotenals were formed. Using HPLC analysis on a RP-C30 column, the decrease of the canthaxanthin peak after addition of nickel peroxide and the formation of degradation prod-

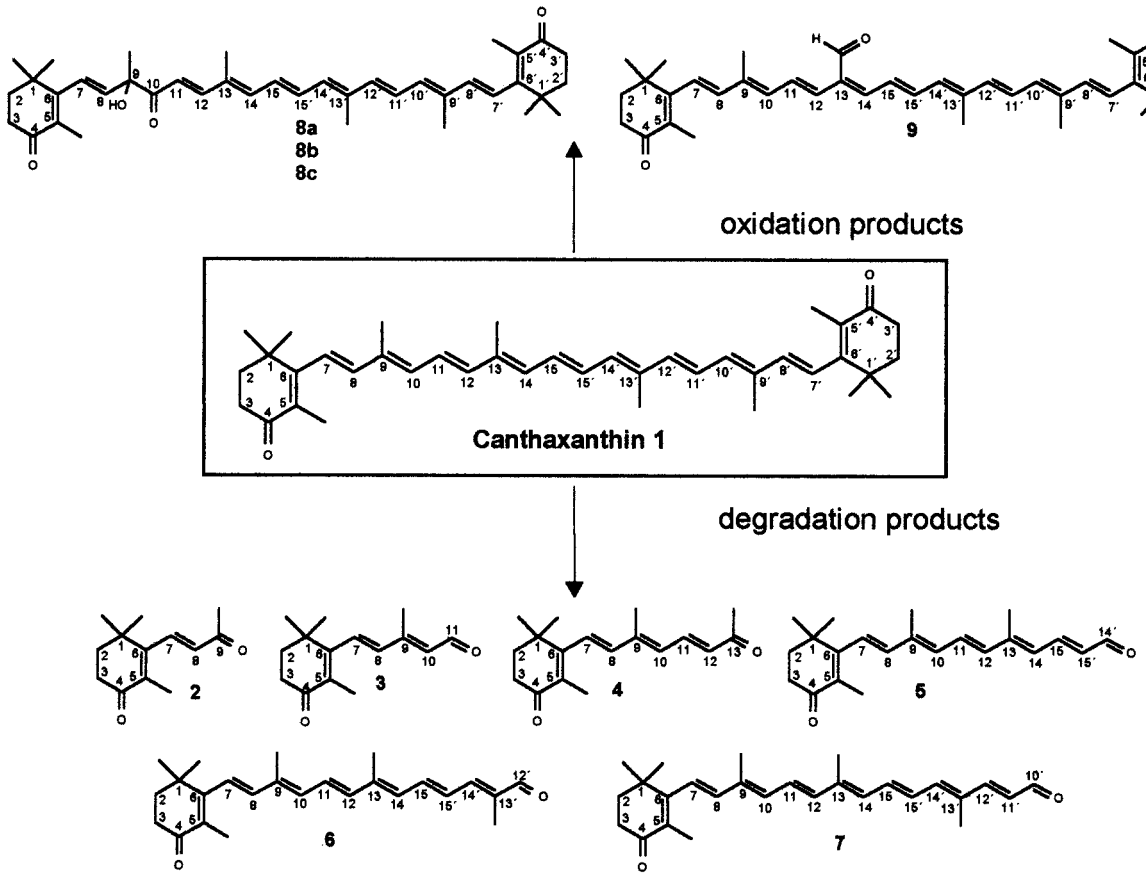

Figure 2. Oxidation and degradation products formed by nickel peroxide treatment of canthaxanthin (**1**).

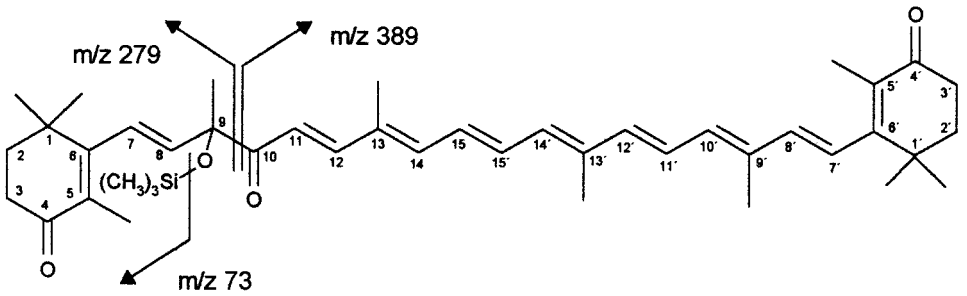

Figure 3. MS fragmentation pattern for silylated compounds **8a**−**8c**.

Table 2. ^{1}H NMR Spectral Data for Ketols 8a−8c[a]

proton	**8a** δ (ppm), signal, J (Hz)	**8b** δ (ppm), signal, J (Hz)	**8c** δ (ppm), signal, J (Hz)
H7′	6.27, d, 16	6.29, d, 16 (6.27 + 0.02)	6.29, d, 16
H8′	6.36, d, 16	6.89, d, 16 (6.36 + 0.53)	6.38, d, 16
H10′	6.28, d, 12	6.21, d, 11.5 (6.28−0.07)	6.34, d, 11.5 (6.28 + 0.05)
H11′	6.75, dd, 15/11.5	6.80, dd, 15/11.5 (6.75 + 0.07)	6.74, dd, 15/11.5
H12′	6.43, d, 15	6.36, d, 15.5 (6.43−0.07)	6.96, d, 15.5 (6.43 + 0.53)
H14′	6.32, d, 12	6.30, d, 12	6.18, d, 12 (6.32−0.13)
H15′	6.86, m	6.86, m	7.01, dd, 15/11.5 (6.86 + 0.16)
H15	6.65, m	6.65, m	6.58, m (6.65 − 0.07)
H14	6.65, m	6.65, m	6.65, m
H12	7.58, d, 15	7.58, d, 15	7.58, d, 15
H11	6.39, d, 15	6.39, d, 15	6.39, d, 16
H8	5.75, d, 16	5.75, d, 16	5.75, d, 16
H7	6.46, d, 16	6.46, d, 16	6.46, d, 16

[a] In parentheses, theoretical changes in chemical shifts are compared to the all-*trans* isomer **8a**, according to Englert (1981).

ucts was monitored over the whole reaction time. HPLC analyses revealed a rapid conversion of canthaxanthin during the first hours (cf. Figure 1) and the generation of considerable amounts of apocarotenoids. Prolonged reaction time decreased the concentration of apocarotenoids and favored the formation of volatile cleavage products. Upon prolonged peroxide treatment of **1**, a whole series of not further characterized volatile breakdown products with 9, 10, 13, and 15 carbon atoms (apparent molecular weight: 138, 152, 164, 166, 188, 204, 206, 232) were detected by GC−MS.

To obtain sufficient amounts of the target apocarotenoids, the oxidation was stopped after 5 h by removing nickel peroxide by filtration over Celite. After concentration, the reaction mixture was fractionated by flash chromatography (Still et al., 1978), and the major products were finally purified by analytical HPLC. In this way, six cleavage products and four oxidation products of canthaxanthin have been obtained (cf. Figure 2). NMR spectral data of the degradation products showed the characteristic signals of a 4-oxo-trimethylcyclohexenyl ring system. Based on one- and two-dimensional NMR spectra as well as MS and UV data, the cleavage products were unambiguously identified as 4-oxo-β-ionone (**2**), (7*E*,9*E*)-4-oxo-β-apo-11-carotenal (**3a**), (7*E*,9*Z*)-4-oxo-β-apo-11-carotenal (**3b**), 4-oxo-β-apo-13-carotenone (**4**), 4-oxo-β-apo-14′-carotenal (**5**), 4-oxo-β-apo-12′-carotenal (**6**), 4-oxo-β-apo-10′-carotenal (**7**). In addition to breakdown products **2**−**7**, several oxygenated canthaxanthin derivatives were also obtained. For three of the oxidation products, a molecular weight of 596 was determined by EI−MS, indicating an incorporation of two oxygen atoms into the canthaxanthin molecule. From the NMR spectral data, oxidation of a double bond with the subsequent formation of a ketol function was apparent.

To determine the exact oxidation site, each of the isomers was treated with MSTFA, and the derivatives obtained were then reanalyzed by EI−MS. In each case, the mass spectra showed prominent peaks at *m*/*z* 668, 389, 279, and 73, which indicated a 9-hydroxy-10-oxo substitution pattern (Figure 3). Since the ^{1}H NMR spectral data of compounds **8a**−**c** were almost identical, they were obviously stereoisomers. On the basis of the NMR data and changes in chemical shifts for protons 7′, 8′, 10′, 11′, 12′ and protons 10′, 12′, 14′, 15′, 15, respectively, the isomers were identified as all-trans (**8a**), 9′-cis (**8b**), and 13′-cis (**8c**) isomers of 9,10-dihydro-9-hydroxy-10-oxo-canthaxanthin (cf. Table 2). Moreover, an oxidation product with a molecular weight of 578 was formed in smaller amounts. From the ^{1}H NMR spectral data, the presence of an aldehyde function was apparent. Since the signal for one methyl group (most likely CH_3−C20) was missing, the oxidation product was tentatively identified as (9′*Z*)-canthaxanthin-20-al (**9**). The tiny amounts of product isolated did not allow confirmation of the suggested structure by two-dimensional NMR.

This study has confirmed earlier reports of Liaaen-Jensen and Hertzberg (1966), Buchecker et al. (1974), and Hohler (1986) that nickel peroxide allows a gentle oxidation of carotenoids. As cleavage products of the 4-oxo-carotenoid **1**, a series of apocarotenals and apocarotenones **2**−**7** have been isolated and characterized. The cleavage products **3a**, **3b**, **4,** and **7** are described here for the first time. Further reaction products included the ketol derivatives **8a**−**c** as well as the tentatively identified canthaxanthin-20-al (**9**). Under the reaction conditions, no oxidation products of the double bonds in the cyclic end group or in the α,α'-positions of the carbonyl groups could be detected. Chromatographic and spectral data are provided, which will facilitate identification of these compounds in natural tissues.

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