Pergamon

Tetrahedron Letters, Vol. 38, No. 45, pp. 7853-7856, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10097-1

Oxidation of Carotenoids - I. Dihydrooxepin Derivatives as Products of Oxidation of Canthaxanthin and β,β-Carotene

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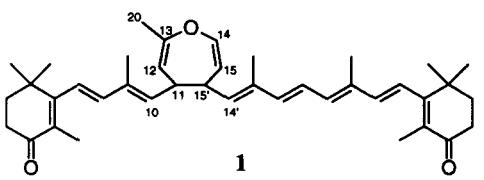
Abstract: Dihydrooxepin derivatives **1** and **2** were obtained by oxidation of canthaxanthin with *m*-CPBA and of β,β-carotene with molecular oxygen, respectively. © 1997 Elsevier Science Ltd.

Carotenoids are reported to have a number of beneficial effects for human health, including vitamin A activity, antioxidative and anticancer properties.[1] The enhancement of gap junctional communication by canthaxanthin and β,β-carotene has been demonstrated in cells in culture, and may provide an explanation for the reported effects of these carotenoids in reducing the risk of some types of cancer.[2]

β,β-Carotene is one of the most widespread natural carotenoids and occurs in a large number of vegetables and fruits, and also in bacteria and fungi. Canthaxanthin is the major pigment in the edible mushroom, *Cantharellus cibarius* Fr., and is found in considerable amounts in fish, crustaceans, bacteria, blue-green algae (Cyanobacteria), and bird plumage.[3]

During our investigations of the oxidation of carotenoids *in vitro*, compound **1**, for which we propose the trivial name 11,15'-dihydrooxepin-canthaxanthin, has been isolated as a major product from the oxidation of canthaxanthin with *m*-chloroperbenzoic acid (*m*-CPBA).[4] An analogous product **2**, named as 11,15'-dihydrooxepin-β,β-carotene, has been obtained as a product from the oxidation of β,β-carotene with molecular oxygen at atmospheric pressure.[5]



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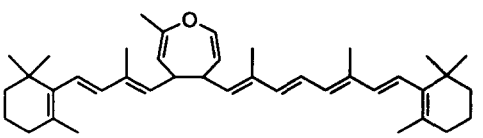

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The possibility must be considered, that such compounds are also formed in human tissues under physiological conditions.

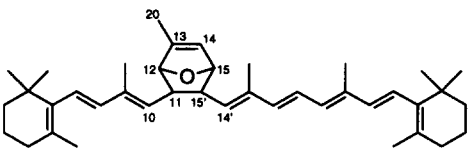
The structure elucidation of **1** and **2** was carried out by a combination of spectroscopic methods. Main characteristics of the UV/Vis spectra were the λ_{max} of 348 nm (**1**, methanol) and 331 nm (**2**, hexane), suggesting a chromophore of 5-6 (**1**), and 4-5 (**2**) conjugated double bonds[6], respectively. The conjugated electronic system of **1** and **2** is therefore considerably shortened compared to that of the original canthaxanthin and β,β-carotene.

Mass spectra showed molecular masses of 580 (**1**) and 552 (**2**), respectively, indicating an uptake of one oxygen atom per carotenoid molecule. Furthermore, both compounds displayed $(M-43)^+$ peaks in the EI mass spectra which are assumed to result from the loss of CH_3-C$^{\bullet}$=O fragments from the oxidised sites of the molecules. In **2** no other oxygen atoms are available to form this radical. From these experimental data we concluded that the oxidised sites of **1** and **2** are located within the polyene chain and that **1** and **2** are likely to be structurally related.

These conclusions are supported by NMR analysis which gave evidence of unchanged carotenoid end groups. Chemical shifts and coupling constants of these six-membered rings were in agreement with those of unchanged canthaxanthin and β,β-carotene. A sequence of 2D NMR experiments, including H,H-COSY, T-ROESY, HMQC, and HMBC experiments, established the constitution of the oxidised site, i. e. the seven-membered dihydrooxepin ring. Evidence was provided by a number of $^3J_{CH}$ couplings observed as cross peaks in the HMBC experiment.[7] Significantly, a C-13/H-14 coupling could be detected in a $^3J_{CH}$-optimised HMBC experiment giving further indication of the ether linkage between C-13 and C-14. Also, the ^{13}C chemical shifts of C-12 and C-15 are in the range of 100-110 ppm[8,9] which is in agreement with chemical shifts found in other dihydrooxepin derivatives[10].

The formation of dihydrooxepin derivatives by thermal rearrangement of diallylic epoxides has been observed previously.[11] Therefore it is likely that **1** and **2** represent rearrangement products of canthaxanthin-13,14-epoxide and β,β-carotene-13,14-epoxide, respectively.

Recently, constitution **3** was assigned to a compound isolated as a product of the oxidation of β,β-carotene induced by the free-radical inducer 2,2'-azobis-(2,4-dimethylvaleronitrile) (AMVN).[12]



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In our opinion, the published spectroscopic data, which are identical with our data for **2**, do not correspond to the proposed constitution **3**. Based on data for oxygen-bridged six-membered ring derivatives[13], the ^{13}C chemical shifts of C-12 and C-15 for constitution **3** should be around 80 ppm which is in disagreement with the experimentally determined values of 100-110 ppm.

In addition, a number of $^3J_{CH}$ couplings, including C-15/H-12, C-12/H-15, C-14/H-12, C-12/H-14 and C-14/H-20, respectively, and the $^4J_{HH}$ coupling H-14/H-20 would be expected to appear as distinct cross peaks in NMR spectra of compound **3**, but these signals were not observed.

We therefore assume that the compound isolated by Yamauchi[12] and assigned constitution **3** was in fact the dihydrooxepin derivative **2**.

ACKNOWLEDGEMENT

We thank F. Hoffmann-La Roche Ltd. for scientific samples and financial support, and the Swiss National Science Foundation for financial support. M. Z. is grateful to Dr. H. Schlichterle-Cerny for technical assistance in the canthaxanthin work.

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4. 1.5 g crystalline canthaxanthin, 2 g ground sodium *m*-chlorobenzoate, and 2 g 6-K-18 crown ether were dissolved in 150 ml CH_2Cl_2, and 1.5 g *m*-CPBA in 15 ml *t*-BuOMe was added at -20 °C. After 30 h, the solution was washed with 5 % Na_2SO_3, dried over Na_2SO_4, diluted with 250 ml hexane and directly submitted to open-top CC with MgO/Celite 1:2 as stationary phase. Compound **1**, the main compound of the first fraction collected with hexane/acetone 95:5, was purified by preparative HPLC, first on a 250 x 10 mm Nucleosil® 120-5 C_{18} column with methanol/H_2O 96:4, and secondly on a 250 x 10 mm Nucleosil® 100-5 CN column with hexane/*t*-BuOMe 7:3 as mobile phase. The detection was performed at 350 nm by means of a HP 1100® photodiode array detector.
5. 500 mg of crystalline β,β-carotene was dissolved in 400 ml toluene and stirred for 4 hours in darkness at 36-38 °C, with oxygen bubbling through the solution. The reaction was stopped by evaporating the solvent *in vacuo*. In-chain oxidation products of β,β-carotene were obtained selectively by pre-separating the reaction mixture by open-top CC on MgO/Celite 1:2 as stationary phase and hexane and hexane/*t*-BuOMe mixtures as eluents. Isolation and final purification of single in-chain oxidation products were performed in multiple steps by HPLC, on a 250 x 10 mm Nucleosil® 100-5 CN column and hexane or hexane/ethyl acetate mixtures as mobile phases. The detection was performed at 330 nm by means of a Waters 990® photodiode array detector.
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7. $^3J_{CH}$ couplings C-15'/H-10, C-10/H-15', C-11/H-14', and C-14/H-11
8. 11,15'-Dihydrooxepin-canthaxanthin (**1**): (25 mg, 2 % yield), yellow solid, UV/Vis λ_{max} 348 nm (methanol), 338 nm (hexane/*t*-BuOMe 7:3); HR-EIMS, m/z 580.3914 (M^+, calc. 580.3916), 537.3727 (M^+-C_2H_3O, calc. 537.3733); EIMS, m/z (% rel. int.); 580 (M^+, 70), 565 (4), 537 (20), 523 (4), 401 (100); ^{1}H NMR (500 MHz, $CDCl_3$), δ 1.15 (6H, s, H-16, H-17), 1.18 (6H, s, H-16', H-17'), 1.80 (3H, s, H-19), 1.82 (3H, s, H-18), 1.82 (3H, s, H-20'), 1.83 (2H, ψt, J≈6.5, H-2), 1.83 (3H, s, H-20), 1.84 (2H, ψt, J≈6.5, H-2'), 1.85 (3H, s, H-18'), 1.95 (3H, s, H-19'), 2.47 (2H, ψt, J≈6.5, H-3'), 2.48 (2H, ψt, J≈6.5, H-3), 3.30 (1H, dψt J=9.2, ≈6, 5.4, H-15'), 3.32 (1H, dψt, J=9.2, ≈6, 5.9, H-11), 4.65 (1H, dd, J=7.3, 5.4, H-15), 4.72 (1H, d, J=5.9, H-12), 5.43 (1H, d, J=9.2, H-14'), 5.50 (1H, d, J=9.2, H-10), 6.08 (1H, d, J=16.3, H-7), 6.17 (1H, d, J=16.3, H-8), 6.18 (1H, d, J=11.3, H-10'), 6.19 (1H, d, J=16.3, H-7'), 6.22 (1H, d, J=7.3, H-14), 6.31 (1H, d, J=15.0, H-12'), 6.32 (1H, d, J=16.3, H-8'), 6.49 (1H, dd, J=15.0, 11.3, H-11') ppm (Hz); ^{13}C NMR (125 MHz, $CDCl_3$, ^{1}H decoupled, *: may be interchanged with corresponding signal) δ 12.45* (C-19'), 12.49* (C-19), 12.81 (C-20'), 13.65 (C-18), 13.77 (C-18'), 21.84 (C-20), 27.44 (C-17),

27.54 (C-16), 27.60 (C-16', C-17'), 34.26 (C-3), 34.26 (C-3'), 35.67* (C-1), 35.70* (C-1'), 37.31* (C-2), 37.36* (C-2'), 41.86 (C-11), 42.41 (C-15'), 106.19 (C-12), 108.73 (C-15), 123.05 (C-7), 123.26 (C-11'), 123.66 (C-7'), 129.62* (C-5'), 129.68* (C-5), 132.33 (C-9), 133.23 (C-13'), 133.97 (C-9'), 134.01 (C-10'), 136.96 (C-14'), 137.45 (C-10), 139.32 (C-12'), 140.89 (C-8), 141.23* (C-8'), 141.27 (C-14), 151.29 (C-13), 161.34 (C-6'), 161.53 (C-6), 199.32 (C-4'), 199.39 (C-4) ppm.

9. 11,15'-Dihydrooxepin-β,β-carotene (**2**): (approx. 1 mg, 0.002 % yield), yellowish film; UV/Vis λ_{max} 331 nm (hexane); LC-MS, APCI⁻, m/z (% rel. int.); 552 (M^-, 100); EIMS m/z (% rel. int.); 552 (M^+, 100), 509 (30); ^{1}H NMR (500 MHz, $CDCl_3$), δ 1.00 (6H, s, H-16, H-17), 1.03 (6H, s, H-16', H-17'), 1.45 (2H, m, not resolved, H-2), 1.47 (2H, m, not resolved, H-2'), 1.60 (2H, m, not resolved, H-3), 1.62 (2H, m, not resolved, H-3'), 1.68 (3H, broad d, J=0.3, H-18), 1.72 (3H, broad d, J=0.6, H-18'), 1.78 (3H, d, J=1.1, H-19), 1.82 (3H, d, J=1.2, H-20'), 1.84 (3H, s, H-20), 1.94 (3H, d, J=0.9, H-19'), 1.99 (2H, m, not resolved, H-4), 2.02 (2H, m, not resolved, H-4'), 3.26 (1H, m, not resolved, H-15'), 3.29 (1H, m, not resolved, H-11), 4.66 (1H, dd, J=7.6, 5.9, H-15), 4.75 (1H, d, J=5.8, H-12), 5.36 (1H, dd, J=9.4, 1.1, H-10), 5.39 (1H, d, J=9.2, H-14'), 5.96 (1H, AB spin system, J=16.0, H-7), 6.00 (1H, AB spin system, J=16.0, H-8), 6.07 (1H, broad d, J=11.3, H-10'), 6.09* (1H, AB spin system, J=16.6, H-7'), 6.14* (1H, AB spin system, J=16.6, H-8'), 6.21 (1H, dd, J=7.58, 0.9, H-14), 6.25 (1H, d, J=15.2, H-12'), 6.50 (1H, dd, J=15.2, 11.3, H-11') ppm (Hz); ^{13}C NMR (125 MHz, $CDCl_3$, ^{1}H decoupled; values from HMQC experiment, no quaternary carbons) δ 12.9 (C-19'), 13.0 (C-19), 13.2 (C-20'), 19.5 (C-3, C-3'), 21.9 (C-18), 22.0 (C-18'), 22.2 (C-20), 29.2 (C-16, C-17, C-16', C-17'), 33.2 (C-4), 33.3 (C-4'), 39.9 (C-2, C-2'), 42.4 (C-11), 42.6 (C-15'), 107.3 (C-12), 109.6 (C-15), 125.3 (C-7), 126.4 (C-7'), 130.9 (C-10'), 134.0 (C-10), 136.3 (C-14), 137.9 (C-8), 137.5 (C-12', uncertain), 138.0 (C-8'), 141.3 (C-14) ppm.
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(*Received in Germany* 16 *July* 1997; *accepted* 8 *September* 1997)