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Oxidation of Carotenoids - II: Ozonides as Products of the Oxidation of Canthaxanthin

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Abstract: As reaction products of canthaxanthin (β,β-carotene-4,4'-dione) with *m*-chloroperbenzoic acid and O_2 two derivatives **1** and **2** with an ozonide moiety in the polyene chain have been isolated. They were identified by UV/Vis-, LC/MS- and one and two dimensional NMR analysis.

Keywords: Carotenoids; Oxidation; Oxygen; Ozonides.

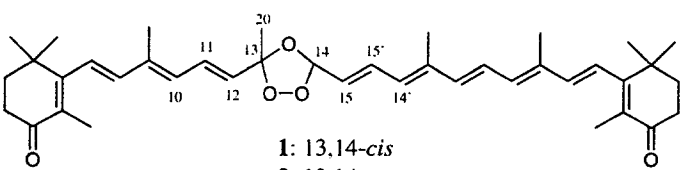
Introduction

Carotenoids are widely distributed in plants, animals, bacteria, fungi, and archea. They are reported to have a number of beneficial effects on human health, including vitamin A activity, anticancer and antioxidant properties[1].

In view of the medical importance of the antioxidant properties the investigation of *in vitro* oxidation of the carotenoids is therefore of major interest.

In a previous communication[2] we have reported the isolation of dihydrooxepins, a new group of carotenoid derivatives, as products of the reaction of canthaxanthin with *m*-chloroperbenzoic acid (*m*-CPBA).

In continuation of these investigations we report in the present publication about the isolation of the ozonides **1** and **2** as products of the oxidation of canthaxanthin (β,β-carotene-4,4'-dione) with *m*-CPBA.



1: 13,14-*cis*
2: 13,14-*trans*

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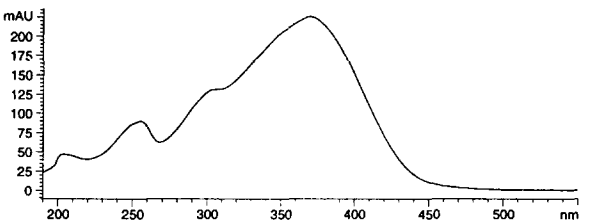
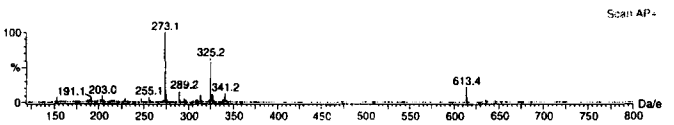
Results and Discussion

The yields of **1** and **2** were strongly dependent on the oxygen content of the solvent. The reaction carried out with solvents saturated with O_2 gave **1** as a main product, whereas **1** was almost absent when degassed solvents were used. Both compounds proved to be relatively unstable and considerable amounts (approx. 70%) were lost during workup at ambient temperatures.

Main characteristic of the UV/Vis spectra of **1**, for which we propose the trivial name canthaxanthin-13,14-ozonide, was the λ_{max} at 370 nm (methanol) without spectral fine structure, suggesting a chromophore of 6-7 conjugated double bonds.[3] The conjugated electronic system is therefore considerably shorter compared to canthaxanthin.



Mass spectra showed a molecular mass of 612 indicating an uptake of three oxygen atoms per carotenoid molecule. The signals at m/z 273, 289, 325 and 341 gave an indication for the cleavage of the polyene chain between C-13 and C-14.



NMR analysis carried out at -20°C exhibited the signals typical for the end groups of canthaxanthin. H, H,H-COSY, HMQC and HMBC NMR Experiments established that the constitution of the polyene chain was unchanged with the exception of the C-13/C-14 bond. Chemical shifts of 5.69 for H-14, 107.5 for C-13 and 104.5 for C-14 are in agreement with the proposed ozonide constitution.[4]

The minor compound **2** exhibited the same UV/Vis and mass spectra as **1**. Compared to **1**, small differences in the ^{1}H-NMR spectrum, especially for the signals of the hydrogens near C-13 and C-14 were observed. Therefore, we conclude that **1** and **2** might be 13,14-*cis/trans* isomers. NOE experiments unfortunately did not supply decisive evidence for determining the relative stereochemistry. However, the chemical shifts of 1.63 for H-20 and 5.69 for H-14 in **1** compared to 1.66 and 5.71 of **2** indicate that **1** is the 13,14-*cis* isomer.

Compound **1** and **2** represent a new group of carotenoid derivatives formed by oxidation and they may be intermediates in the formation of apocarotenoids, formed by oxidation of C_{40}-carotenoids.

In addition it was observed that the reaction of 11,15'-dihydrooxepin-canthaxanthin with O_2 at 37°C gave compounds which exhibited identical chromatographic behaviour and UV/Vis-spectra compared to the ozonides.

Summary

The isolation of two carotenoid derivatives **1** and **2,** containing an ozonide moiety, which were obtained by the oxidation of canthaxanthin with *m*-CPBA and O_2 is described. The hitherto unknown compounds were characterized by UV/Vis, LC/MS and one and two dimensional NMR analysis. Compound **1** and **2** may be intermediates in the formation of apocarotenoids, formed by oxidation of C_{40}-carotenoids.

Experimental

General. All operations were carried out in diffuse daylight or subdued artificial light. UV/Vis were measured after HPLC analysis with a HP 1100® photodiode array detector. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were measured on Bruker DRX-400. LC/APCI/MS were obtained on VG Platform (Micromass).

Isolation of 13,14-*cis*-Canthaxanthin-13,14-ozonide (1) and 13,14-*trans*-Canthaxanthin-13,14-ozonide (2). To a solution of 0.25 g crystalline canthaxanthin, 0.25 g ground sodium-*m*-chlorobenzoate, and 0.25 g 6-K-18 crown ether in 25 ml CH_2Cl_2, a solution of 0.25 g *m*-CPBA in 2.5 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 50 ml hexane and directly submitted to TLC with MgO/kieselgur 1:1 as stationary phase and developped with 20% acetone in hexane. The band, which mainly consisted of **1** and **2** was purified first by TLC on aluminiumoxide with 10% acetone in hexane, then by preparative HPLC, first on a 250 x 10 mm Nucleosil® 120-5 C_{18} column with MeOH/H_2O 96:4, and secondly on a 250 x 10 mm Nucleosil® 100-5 CN column with hexane/*t*-BuOMe 1:1 as mobile phase. The detection was performed at 350 nm. Yields: 1.5 mg (1 %) for **1** and 0.4 mg (0.2 %) for **2**.

Oxidation of 11,15'-dihydrooxepin-canthaxanthin. A solution of 10 µg 11,15'-dihydrooxepin-canthaxanthin in 100 µl *t*-BuOMe, covered with O_2 was reacted at 37°C for 3 h. Analysis of the mixture was performed by HPLC/PDA with a 250 x 4.6 mm Nucleosil® 120-3 C_{18} column and MeOH:H_2O = 96:4 as solvent.

13,14-*cis*-Canthaxanthin-13,14-ozonide (1). yellow solid; UV/Vis λ_{max} 370 nm (MeOH), 368 nm (hexane/*t*-BuOMe 1:1); ^{1}H-NMR (400 MHz, $CDCl_3$), δ = 1.18 (6H, s, H-16,17), 1.19 (6H, s, H-16',17'), 1.63

(3H, s, H-20), 1.84 (3H, s, H-18), 1.85 (2H, *ψt*, J≈6.5, H-2), 1.85 (2H, *ψt*, J≈6.5, H-2'), 1.86 (3H, s, H-18'), 1.98 (3H, s, H-19), 1.98 (3H, s, H-20'), 2.00 (3H, s, H-19'), 2.51 (2H, *ψt*, J≈6.5, H-3), 2.51 (2H, *ψt*, J≈6.5, H-3'), 5.62 (1H, dd, J=15.1, 7.1, H-15), 5.69 (1H, d, J=7.1, H-14), 5.86 (1H, d, J=15.1, H-12), 6.15 (1H, d, J =11.4, H-10), 6.18 (1H, d, J =11.4, H-14'), 6.24 (1H, d, J =16.2, H-7'), 6.25 (1H, d, J =11.4, H-10'), 6.28 (1H, AB, J =16.2, H-7), 6.28 (1H, AB, J =16.2, H-8), 6.34 (1H, d, J =16.2, H-8'), 6.38 (1H, d, J =15.0, H-12'), 6.72 (1H, dd, J=15.0, 11.4, H-11'), 6.83 (1H, dd, J=15.1, 11.4, H-11), 6.92 (1H, dd, J=15.1, 11.4, H-15'); ^{13}C-NMR (100 MHz, $CDCl_3$), δ = 12.5 (C-19, 19', 20'), 14.0 (C-18, 18'), 21.7 (C-20), 27.5 (C-16, 16', 17, 17'), 34.0 (C-3, 3'), 35.0 (C-1, 1'), 36.5 (C-2, 2'), 104.5 (C-14), 107.5 (C-13), 122.0 (C-15), 124.5 (C-7'), 125.0 (C-7), 126.5 (C-11'), 127.0 (C-11), 129.5 (C-5, 5'), 129.5 (C-14'), 131.5 (C-10), 133.5 (C-12), 134.0 (C-10'), 135.0 (C-9'), 135.7 (C-15'), 136.5 (C-9), 138.0 (C-12'), 139.0 (C-13'), 140.5 (C-8), 141.0 (C-8'), 161.0 (C-6, 6'), 199.5 (C-4, 4'); LC/APCI-/MS, m/z (% rel. int.); 580 (M-, 5) 540 (4), 340 (25), 324 (60), 311 (10), 287 (7), 272 (100), 245 (10); LC/APCI+/MS, m/z (% rel. int.); 635 (M+Na, 5), 613 (M+H, 23), 525 (2), 341 (12), 325 (65), 313 (10), 289 (15), 273 (100), 247 (5).

13,14-*trans*-Canthaxanthin-13,14-ozonide (2). yellow solid, UV/Vis λ_{max} 370 nm (MeOH), 368 nm (hexane/*t*-BuOMe 1:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.17 (6H, s, H-16,17), 1.18 (6H, s, H-16',17'), 1.66 (3H, s, H-20), 1.82 (3H, s, H-18), 1.84 (4H, *ψt*, J≈6.5, H-2,2'), 1.85 (3H, s, H-18'), 1.97 (3H, s, H-20') 1.99 (3H, s, H-19), 2.00 (3H, s, H-19'), 2.50 (4H, *ψt*, J≈6.5, H-3, 3'), 5.64 (1H, dd, J≈15, 7.6, H-15), 5.71 (1H, d, J=7.6, H-14), 5.78 (1H, d, J=15.1, H-12), 6.16 (1H, d, J =11.4, H-10), 6.16 (1H, d, J =11.4, H-10'), 6.19 (1H, d, J =11.4, H-14'), 6.24 (1H, d, J =16.2, H-7'), 6.26 (1H, AB, J =16.2, H-7), 6.26 (1H, AB, J =16.2, H-8), 6.32 (1H, d, J =16.2, H-8'), 6.40 (1H, d, J =15.0, H-12'), 6.71 (1H, dd, J=15.0, 11.4, H-11'), 6.89 (1H, dd, J=15.1, 11.4, H-11), 6.93 (1H, dd, J≈15, 11.4, H-15'); LC/APCI-/MS, m/z (% rel. int.); 580 (M-, 5) 540 (4), 340 (25), 324 (60), 311 (10), 287 (7), 272 (100), 245 (10); LC/APCI+/MS, m/z (% rel. int.); 635 (M+Na, 5), 613 (M+H, 23), 525 (2), 341 (12), 325 (65), 313 (10), 289 (15), 273 (100), 247 (5).

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