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Isolation of Capsanthone ((all-*E*,3*R*,5'*R*)-3-Hydroxy- β , κ - carotene-3',6'-dione) from Paprika (*Capsicum annuum*)

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Abstract. From different species of paprika (*Capsicum annuum*) capsanthone (**1**) was isolated and characterized as (all-*E*,3*R*,5'*R*)-3-hydroxy- β , κ -carotene-3',6'-dione.

Introduction

The occurrence of capsanthone (**1**) and other keto carotenoids such as capsorubone, cryptocapsone *etc.* in the anthers of the flowers of different species of *Aesculus* (horse chestnut) has been reported a long time ago [1]. The constitution of the isolated non-crystalline compound was postulated as 3-hydroxy- β , κ -carotene-3',6'-dione, based on UV/VIS spectra, chemical and chromatographic properties, yet the stereochemistry at C(3) and C(5') remained unknown.

On the other hand (all-*E*,3*R*,5'*R*)-**1** has been prepared by Oppenauer oxidation of natural and synthetic capsanthin (**2**) [2][3].

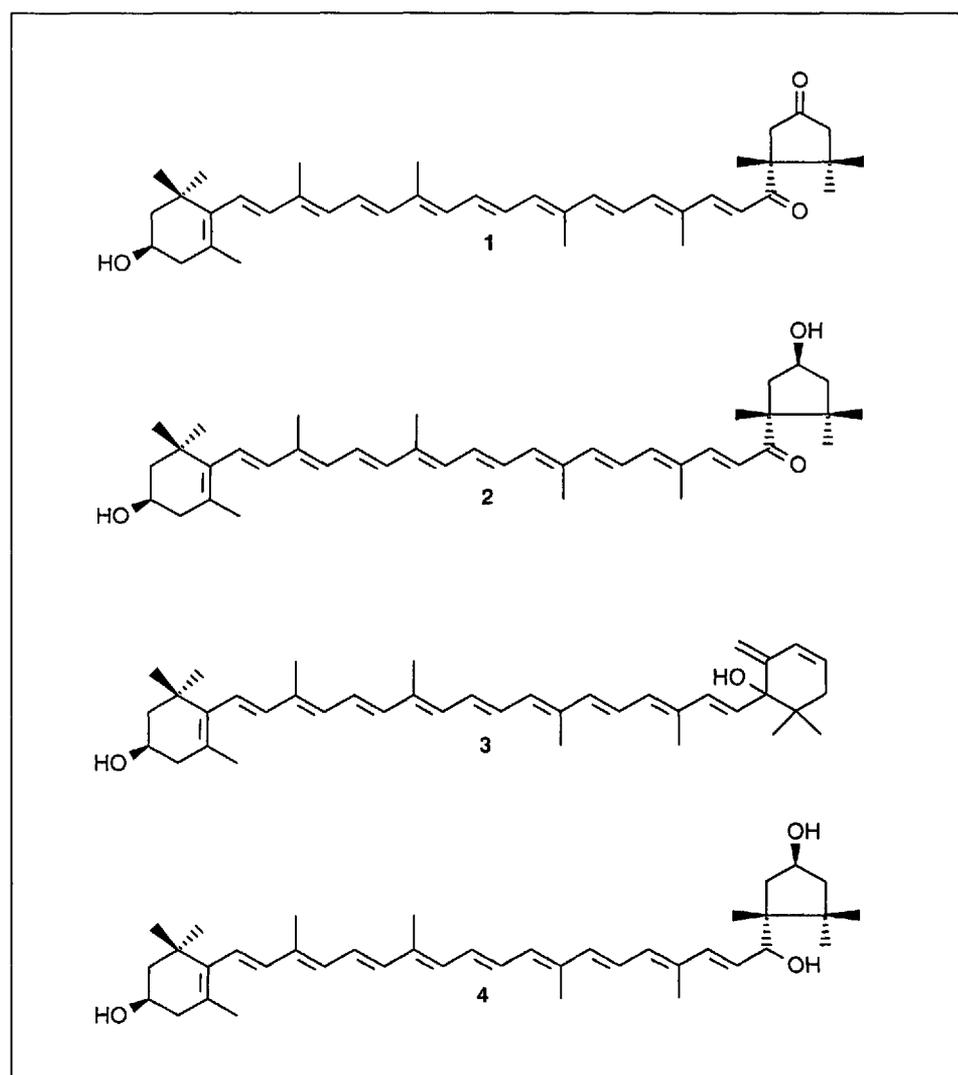
During our investigations of different species of paprika (*Capsicum annuum*) novel carotenoids with the 7-oxabicyclo[2.2.1]heptyl(3,6-epoxycyclohexyl) end group such as cucurbitaxanthin A and B, 3,6-epoxycapsanthin and cycloviolaxanthin have been isolated and characterized [4]. Furthermore, in black paprika (*C.a. var. longum nigrum*) nigroxanthin (3,3',4'-didehydro- β , γ -carotene-3,6'-diol), which contains a hitherto unknown end group, was identified [5]. During the isolation procedure of this new carotenoid, HPLC investigations showed that the 'nigroxan-

thin fraction' contained another carotenoid in a significant amount.

In the present paper we report on the isolation and structure elucidation of capsanthone (**1**) from different species of paprika.

Results and Discussion

From one of the fractions of the methanolic extract of 22 kg of black paprika (*Capsicum annuum var. longum nigrum*) 295 mg solid material was precipitated by benzene/hexane (see *Exper. Part*). The precipitate was separated on a CaCO₃ column (benzene/hexane 2:3) and gave a fraction of 5 mg which contained nigroxanthin (**3**) as the major carotenoid. This fraction was subjected to a second column chromatography on CaCO₃ (hexane/acetone 100:3) to give nigroxanthin (**3**) and capsanthone (**1**). The latter was crystallized from benzene/hexane to give 0.8 mg of red crystals. HPLC investigations showed a purity of > 98% and co-chromatography of the isolated with the semi-synthetic **1** showed accordance with one another. The UV/VIS spectrum with a maximum at 493 nm (in benzene) was identical with the one in [2d][2e]. Reduction of **1** with NaBH₄ gave 3,3',6'-trihydroxy- β , κ -carotene (**4**) whose UV/VIS spectrum exhibited, as expected a shape with a fine structure and a hypsochromic



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shift (434, 457, 487 nm in benzene). The MS spectra of the semi-synthetic [2e][3b] and natural capsanthone (**1**) are in excellent agreement exhibiting a molecular ion at m/z 582 and strong characteristic signals at 476 ($M-106$), 125, 105, 91 and 83. Acetylation of **1** gave **1a** (= **1** with AcO at C(3)) whose MS spectra showed the molecular ion at m/z 624 which is in accordance with the monoacetate of **1**.

Comparing the $^1\text{H-NMR}$ spectra of natural and semi-synthetic capsanthone (**1**), identical signal patterns except for

minimal deviations in chemical shift and line width values, caused by the extreme difference of the sample concentrations (0.8 mg and 11.0 mg, respectively, in 0.5 ml CDCl_3 each) are observed. This proves the identity of the constitution and the stereochemistry at the double bonds of both compounds.

The $^1\text{H-}$ and $^{13}\text{C-}$ signals of the capsanthone (**1**) end groups have been assigned previously [6][7] and later additional proton data, but without detailed spectral analysis of the olefinic range, have been re-

ported [3b]. A complete assignment of the $^1\text{H-}$ and $^{13}\text{C-}$ resonances in capsanthone (**1**) has been carried out and the spectroscopic results for the semi-synthetic compound are listed in the Table.

The $\delta(^1\text{H}, ^{13}\text{C})$ chemical shift and $J_{\text{H,H}}$ coupling constant values of the end groups correspond to those of the literature quoted above. To assign the $^1\text{H-}$ signals of the olefinic chain, a double-quantum-filtered H,H-COSY spectrum was performed, in which all relevant cross peaks were visible. The 'weak coupling' condition $\Delta\delta \gg J$ is fulfilled for H-C(10), H-C(11) and H-C(12); their spin system can therefore be analyzed as a first-order system. $^3J_{10/11} = 11.5$ Hz and $^3J_{11/12} = 15.2$ Hz are characteristic for a *trans*-arrangement. More complex relationships are found for H-C(14), H-C(15), H-C(14'), and H-C(15'): Under the influence of the different end groups a spin system is formed which may be described as an *ABMX* type. Without simulation methods no informations about coupling constants can be extracted out of the spectrum. Similar relationships occur for the *ABM*-type spin system of H-C(10'), H-C(11'), and H-C(12'). However, their chemical shifts can be extracted from single traces of the COSY spectrum and are given as centres of the corresponding multiplets. The signals of $\text{H}_3\text{C}(19)$, $\text{H}_3\text{C}(20)$, $\text{H}_3\text{C}(19')$, and $\text{H}_3\text{C}(20')$ can be identified by means of their coupling with in-chain H-atoms.

For the assignment of the carbon resonances a C,H-shift correlation and a long-range C,H-shift correlation (COLOC) experiment was performed. With the exception of the $^{13}\text{C-}$ signals of C(19), C(20), C(19'), and C(20'), which show fused cross peaks, all resonances, including those of quaternary carbons, could be assigned without doubt.

As mentioned before semi-synthetic capsanthone (**1**) with the (3*R*,5'*R*)-configuration has been prepared previously [3b]. Semi-synthetic and natural **1** showed practically the identical CD spectra with positive maxima at 204, 255, 357 nm and negative maxima at 226, 300, 480 nm.

Therefore the (3*R*,5'*R*)-configuration of natural capsanthone (**1**) is established.

In continuation of these investigations capsanthone (**1**) was also isolated from red spice paprika (*Capsicum annuum* var. *longum Kalocsaense*) (see *Exper. Part*) and showed identical spectroscopical data.

In conclusion our investigations prove the structure of natural capsanthone (**1**) as (all-*E*,3*R*,5'*R*)-3-hydroxy- β , κ -carotene-3',6'-dione and show the natural occurrence of **1** in different species of paprika (*Capsicum annuum*).

Table. $^1\text{H-}$ and $^{13}\text{C-NMR}$ Data for Semi-Synthetic Capsanthone (**1**)

C-atom	$\delta^1\text{H}$ [ppm] ($J_{\text{H,H}}$ [Hz])	$\delta^{13}\text{C}$ [ppm]
1	–	37.11
2	1.48 <i>ax</i> , 1.76 <i>eq</i> ($J_{\text{gem}} = 11.9$)	48.42
3	4.00	65.04
4	2.04 <i>ax</i> , 2.39 <i>eq</i> ($J_{\text{gem}} = 16.7$)	42.55
5	–	126.28
6	–	137.72
7	6.11 (<i>AB</i> system)	125.91
8	6.11 (<i>AB</i> system)	138.40
9	–	136.20
10	6.15 ($J_{10,11} = 11.5$)	131.18
11	6.65 ($J_{11,10} = 11.5$, $J_{11,12} = 15.2$)	125.62
12	6.36 ($J_{12,11} = 15.2$)	137.32
13	–	137.79
14	6.26 (<i>ABMX</i> system, X-part) ^{a)}	132.31
15	6.69 (<i>ABMX</i> system, M-part) ^{a)}	131.90
16	1.07	28.72
17	1.07	30.25
18	1.73	21.60
19	1.96	12.80 ^{b)}
20	1.98	12.71 ^{b)}
1'	–	41.11
2'	2.24, 2.28 (<i>AB</i> system)	52.40
3'	–	216.48
4'	2.08 <i>ax</i> , 3.09 <i>eq</i> ($J_{\text{gem}} = 18.7$)	48.21
5'	–	55.82
6'	–	201.49
7'	6.47 ($J_{7,8'} = 15.0$)	119.74
8'	7.39 ($J_{8,7'} = 15.0$)	148.23
9'	–	133.35
10'	6.60 (<i>ABM</i> system, A-part) ^{a)}	141.76
11'	6.59 (<i>ABM</i> system, B-part) ^{a)}	123.93
12'	6.54 (<i>ABM</i> system, M-part) ^{a)}	142.61
13'	–	135.79
14'	6.36 (<i>ABMX</i> system, A-part) ^{a)}	135.68
15'	6.63 (<i>ABMX</i> system, B-part) ^{a)}	129.60
16'	1.01	25.24
17'	1.24	24.81
18'	1.39	19.80
19'	1.96	12.67 ^{b)}
20'	1.97	12.77 ^{b)}

^{a)} Chemical shifts correspond to the center of the multiplet.

^{b)} Assignments may be interchanged.

Experimental Part

General. – HPLC: Gynkotek pump Model 300 B with Gynkotek Gradient Former, Detector: Waters-991, Photo diode array. Column: 250 x 4.6 mm i.d., Chromsyl C₁₈, 6 mm, endcapped. Mobile phase: eluent A: 12% H₂O in MeOH, eluent B: MeOH, eluent C: acetone/MeOH 1:1. Gradient program: 0–2 min: 100% A, – 16 min: to 50% B, – 24 min: to 100% B, – 30 min: 100% B (linear steps). UV/VIS: Beckman DU-65. CD: Jobin-Yvon Dichrograph-6 in EtOH at r.t. MS: Jeol JMS-01-SG-2. NMR: Bruker AM 400 (¹H: 400.14 MHz, ¹³C: 100.61 MHz); chemical shifts (δ) in ppm (relative to the solvent signal), coupling constants (J) in Hz. CC: CaCO₃ (Biogal, Hungary). After development the columns were extruded and cut into pieces. TLC: Silica F₂₅₄ (Merck 5554).

Isolation. – Ripe paprika (*Capsicum annum* var. *longum nigrum*), freed from their seeds and stem (22 kg of fresh weight), were used for extraction. The paprika was blended with MeOH and ca. 1% CaCO₃. The mixture was allowed to stand in MeOH for dehydration for 20 h. After filtration, the filter cake was extracted twice with MeOH. The combined MeOH extracts were transferred to a separatory funnel, diluted with Et₂O, washed free of MeOH with H₂O, dried over anh. Na₂SO₄, evaporated *i.v.* to about half-volume, and saponified with 30% KOH/MeOH at r.t. for 18 h. The ethereal soln. was washed free of alkali. On addition of benzene/hexane 1:2 a precipitate (2.19 g) was received. The mother liquor was evaporated *i.v.* and dissolved in benzene. On addition of hexane 295 mg crystalline red solid was received. The precipitate was submitted to CC: 10 columns 6 x 30 cm, benzene/hexane 2:3. Picture after development: 1 mm brick red, 2 mm ochre, 3 mm violet (3,6-epoxycapsanthin), 2 mm yellow (cucurbitaxanthin B + cucurbitachrome B), 1 mm brick red, 2 mm pale yellow, 7 mm intermediate zone, 3 mm pale ochre (3 + 1, 5 mg), 2 mm intermediate zone, 15 mm yellow (cucurbitaxanthin A), 2 mm intermediate zone, 15 mm yellow (cycloviolaxanthin), 20 mm intermediate zone, 3 mm pale yellow. The zone containing 1 and 3 was subsequently submitted to a second CC: 2 columns 6 x 30 cm, 3% acetone in hexane. Picture after development: 1 mm pale yellow, 15 mm yellow 3, 2 mm intermediate zone, 5 mm pink 1, 3 mm intermediate zone, 3 mm pale yellow. After desorption 1 was crystallized (benzene/hexane) to give 0.8 mg of red crystals (m.p. 152–154°), with a purity of 98%, TLC: R_f = 0.65 (benzene/AcOEt/MeOH 7:2:1). Further ethereal extraction of the filter cake resulted in approx. 15 g precipitate containing polar carotenoids [4c]. For the isolation of 1 from red spice paprika (*Capsicum annum* var. *longum Kalocsaiense*) 1 kg (fresh weight) of ripe paprika and the same isolation procedure as described above was used. After saponification the ethereal soln. was washed free of alkali, evaporated to dryness *i.v.*, the residue was dissolved in benzene/hexane and the product was submitted directly to CC: 10 columns 6 x 30 cm, benzene/hexane 3:2. Picture after development: 5 mm brick red, 10 mm violet, 5 mm yellow, 50 mm black red (the three last zones contained capsorubin, karpoxanthin and 2 as a mixture), 3 mm yellow (violaxanthin), 5 mm pale ochre (mixture containing 1), 30 mm yellow (cucurbitaxanthin A), 1 mm violet, 15 mm pale

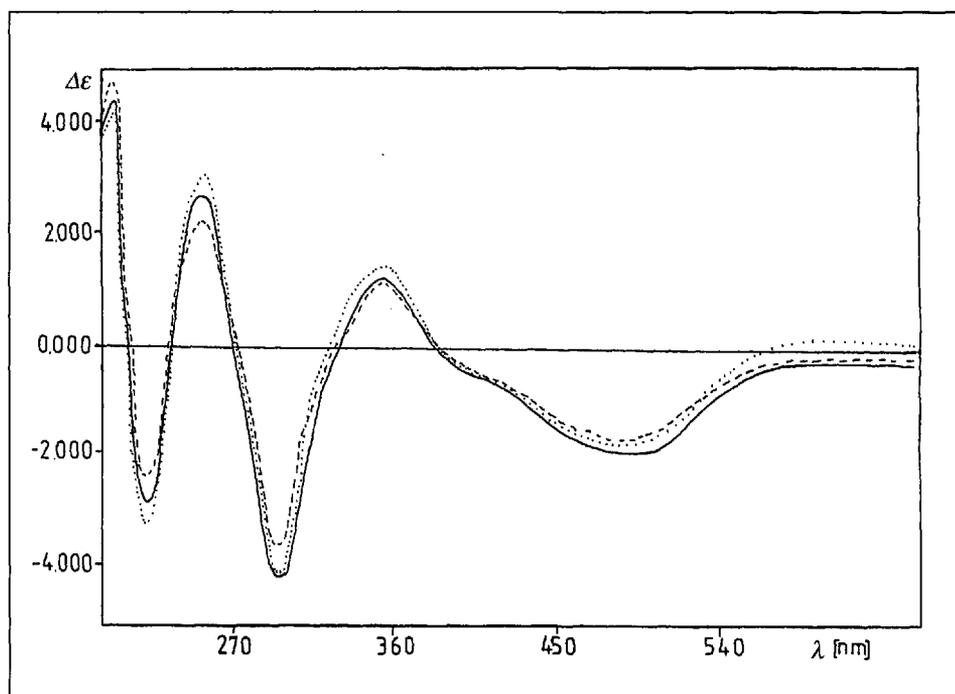


Figure. CD-spectra of natural (3R,5'R)-capsanthone (----), semi-synthetic capsanthone (.....) and fitting of CD-spectra of natural capsanthone to the minimum of semi-synthetic capsanthone at 300 nm (—)

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yellow (zeaxanthin), 10 mm yellow (β -cryptoxanthin). The zone containing 1 was submitted to a second CC: column 3 x 30 cm, acetone/hexane 3:97. Picture after development: 2 mm lemon yellow, 10 mm intermediate zone, 25 mm yellow, 8 mm pink 1, 20 mm intermediate zone, 5 mm ochre. 1 was identified by TLC, HPLC, UV/VIS-spectroscopy and chemical reactions (including co-chromatography with an authentic sample).

Spectroscopical data of 1. UV/VIS (benzene): 493 nm. EI-MS 582 (28, M^+), 476 (30), 145 (11), 125 (45), 119 (53), 106 (15), 105 (51), 91 (68), 83 (100), 69 (32), 55 (62). CD (EtOH, 8.3 x 10⁻⁵ Mol/l): 204.0 (4.80), 226 (-2.38), 254.5 (2.28), 300.0 (-3.45), 356.5 (1.10). ¹H- and ¹³C-NMR cf. the Table.

3,3',6'-Trihydroxy- β , κ -carotene (4). Reduction of 1 with NaBH₄ according to [8]. Crystallization: benzene/hexane; m.p. 157–158°. UV/VIS (benzene): 487, 457, 434 nm. EI-MS: 586 (85, M^+), 129 (44), 119 (74), 109 (61), 106 (57), 105 (58), 91 (100), 73 (53).

Capsanthone monoacetate: Acetylation according to [9]. Crystallization: benzene/MeOH; m.p. 136°. TLC: R_f = 0.90 (benzene/AcOEt/MeOH 7:2:1). UV/VIS (benzene): 493 nm. EI-MS: 624 (85, M^+), 564 (44), 518 (33), 458 (89), 187 (26), 146 (81), 133 (41), 125 (59), 119 (74), 106 (28), 105 (67), 91 (100), 83 (96), 55 (78).

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