Nigroxanthin (3',4'-Didehydro-β, γ-carotene-3,6'-diol), a New Carotenoid Isolated from Paprika (*Capsicum annuum var. longum nigrum*)

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Abstract. From red paprika (*Capsicum annuum var. longum nigrum*) nigroxanthin (**1**) was isolated as a minor carotenoid and, based on its spectral data, identified as (all-*E*)-3',4'-didehydro-β,γ-carotene-3,6'-diol.

Introduction

The different varieties of paprika (*Capsicum annuum*) have been investigated for a long time. It has been established that capsanthin ((3*R*,3'*S*,5'*R*)-3,3'-dihydroxy-β,κ-caroten-6'-one) and capsorubin ((3*S*,5*R*,3'*S*,5'*R*)-3,3'-dihydroxy-κ,κ-caro-tene-6,6'-dione), both of which contain the five membered ring κ-end group, are the most abundant carotenoids in these vegetables [1]. Furthermore, many other carotenoids with interesting structures, especially those with the oxabicyclo-β-end group, have been isolated [2][3]. Recently, the carotenoid composition of the different paprika varieties at different stages of ripening was under investigation [4][5]. This was done in view of the elucidation of

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the biosynthesis of the κ-end group, which still has not been completely established.

During the investigation of the black variety (*Capsicum annuum var. longum nigrum*) 58 peaks were observed by HPLC, and 34 carotenoids were completely or tentatively identified [5]. In this paper, as a continuation of these studies, the isolation of a hitherto unknown carotenoid, the assignment of its constitution and its configuration at the double bonds is reported.

Results and Discussion

During the isolation of cycloviolaxanthin [3], several unknown carotenoids were observed by column chromatography. The compound which was absorbed between cucurbitaxanthin A and B (zone 7 in [3], and peak 30 in Fig. 1 in [5]) on the CaCO₃ column (*Biogal*, Hungary) was further investigated. From this zone, a new carotenoid, for which the name '*Nigroxanthin*' (**1**) is proposed, was isolated and crystallized from benzene/hexane (m.p. 125–127°).

The UV/VIS spectrum (λ_{max}, benzene: 487, 457, and 434 nm, no *cis* peak) shows that the compound contains an (all-*E*)-decaene chromophore. In accordance with that, no reaction took place with LiAlH₄ or HCl/AcOH indicating that no carbonyl or 5,6-epoxy groups are present. The EI-MS

shows the signal for the molecular ion at m/z 566 (100, M^+) which corresponds to $C_{40}H_{54}O_2$. Further characteristic signals can be observed at 548 ($[M - H_2O]^+$), 530 ($[M - 2H_2O]^+$), 474 ($[M - 92]^+$), 119, and 105. Characteristic signals for allenes, acetylenes, carbonyl, carboxyl, and epoxy groups were absent in the IR spectrum. Acetylation gave a crystalline compound with a molecular ion at m/z 608 (100) in the MS corresponding to a monoacetate. The reaction with $(CH_3)_3SiCl/((CH_3)_3Si)_2$ resulted in a mono-trimethylsilyl ether (MS: 638 (M^+)). These derivatives give an indication that nigroxanthin (**1**) contains one *prim.* or *sec.* and one *tert.* OH group.

For the NMR investigations, the compound was once again recrystallized. The HPLC analysis of this purified compound showed a purity of > 98%. The analysis of the ¹H- and ¹³C-NMR spectra of nigroxanthin (**1**) was restricted to the end group signals, as the application of modern techniques is hampered by the small sample concentration. However, larger quantities of deoxylutein II (**2**) and deoxylutein III (**3**) were available to perform the experiments necessary for complete structural elucidation. Taking advantage for their structural relationship to **1**, their data serve for further signal identifications in the spectra of nigroxanthin.

NMR Investigations of both carotenoids **2** and **3** have been already published [6], but as we obtained more detailed spectral informations and found different line assignments in some cases, all NMR spectroscopic data for **1–3** are shown in the *Table*. Not to overload the table, only relevant $^nJ(H,H)$ values are listed. The ¹H and ¹³C resonances of the well-known β-end groups can clearly be assigned on the basis of their chemical-shift values and coupling interactions, and are in agreement with data from [7][8].

The ¹H-NMR resonances for the geminal protons H₂C(2') in the γ-end group are identified due to their chemical shifts of 2.30 and 2.22 ppm. In the H,H-COSY spectrum cross-peaks between H₂C(2') and H–C(3') as well as between H–C(3') and H–C(4') are visible, so that the signals for both olefinic protons can be assigned. Two slightly broadened singlets, with δ values (5.03 and 5.00 ppm) typical for exocyclic olefinic CH₂ protons, correspond to the nuclei H₂C(18'), which may be arbitrarily named H_a and H_b. Their very small coupling interaction, causing signal broadening, is detectable in the COSY spectrum. For deoxylutein II (**2**), it can be verified by 1D NOE difference experiments that the signal appearing at lower field corresponds to H_a (NOE H_a ↔ H–C(4')), and the reso-

Table. *¹H- and ¹³C-NMR Data for* **1**, **2**, *and* **3**

C	¹H 1	2	3	¹³C 1	2	3
1	–	–	–	37.13	37.12	37.12
2	1.77 eq, 1.48 ax	1.77 eq, 1.48 ax	1.77 eq, 1.48 ax	48.46	48.44	48.44
3	4.00, OH: 1.56	4.00, OH: 1.48	4.00, OH: 1.40	65.10	65.09	65.09
4	2.39 eq, 2.05 ax	2.39 eq, 2.02 ax	2.39 eq, 2.04 ax	42.58	42.56	42.56
5	–	–	–	126.16	126.16	126.15
6	–	–	–	137.77	137.77	137.77
7	6.10 (AB)	6.10 (AB)	6.11 (AB)	e)	125.55	125.55
8	6.15 (AB)	6.15 (AB)	6.15 (AB)	138.51	138.51	138.51
9	–	–	–	135.57 a)	135.47 a)	135.63
10	6.19 (10.4)	6.17 (12.2)	6.16 (12.0)	131.32	131.32	131.32
11	6.65 (10.4, 14.8)	6.64 (12.2, 14.9)	6.63 (12.0, 14.9)	124.90 b)	124.87	124.86
12	6.36 (14.8)	6.36 (14.9)	6.36 (14.9)	137.58	137.59	137.59
13	–	–	–	136.44	136.39	136.37
14	6.25 a)	6.25	6.26	e)	132.62 b)	132.62
15	6.64	6.61	6.63	e)	130.10 c)	130.11 a)
16	1.07	1.07	1.07	28.73	28.73	28.72
17	1.07	1.07	1.07	30.26	30.26	30.26
18	1.71	1.74	1.73	21.82	21.61	21.62
19	1.97	1.97	1.97	12.82	12.81	12.81
20	1.97	1.97	1.97	12.82	12.81	12.81
1'	–	–	–	49.81	33.45	34.81
2'	2.30, 2.22 (AB)	1.94, 2.01 (AB)	5.32 (9.4)	29.70	38.52	134.53
3'	5.67 (10.1, 3.7)	5.71 (10.1, 4.7)	5.75 (9.4, 5.1)	e)	127.81	122.10
4'	6.26	6.18	5.60 (5.1)	e)	128.30	117.55
5'	–	–	–	149.32	145.68	137.22
6'	–	2.65 (9.2)	2.20 (10.0)	73.67	55.49	55.72
7'	e)	5.64 (15.5, 9.2)	5.57 (15.1,10.0)	e)	129.24	128.00
8'	e)	6.16 (15.5)	6.12 (15.5)	e)	136.59	135.88
9'	–	–	–	135.66 a)	135.64 a)	135.73
10'	6.13 (10.5)	6.16 (11.8)	6.15 (11.7)	e)	130.66	130.46
11'	6.64 (10.5, 14.8)	6.61 (11.8, 15.0)	6.60 (11.7, 15.0)	124.94 b)	124.98	125.01
12'	6.36 (14.8)	6.34 (15.0)	6.33 (15.0)	137.49	137.28	137.14
13'	–	–	–	136.47 c)	136.51	136.53
14'	6.26 a)	6.24	6.25	e)	132.40 b)	132.35
15'	6.64	6.61	6.63	e)	129.95 c)	129.92 a)
16'	1.13 b)	0.88 a)	0.93	e)	25.33 d)	25.82
17'	1.22 b)	0.90 a)	1.00	e)	28.42 d)	26.66
18'	5.03 Ha, 5.00 Hb	4.88 Ha, 4.81 Hb	1.72	105.09	112.97	22.17
19'	1.97	1.92	1.88	13.22	13.13	13.12
20'	1.97	1.96	1.96	12.76	12.75	12.75

Chemical shift values: δ [ppm] J(H,H) coupling constant values: (J [Hz]).
a)–d) Assignment may be interchanged.
e) Not assigned.

1

2

3
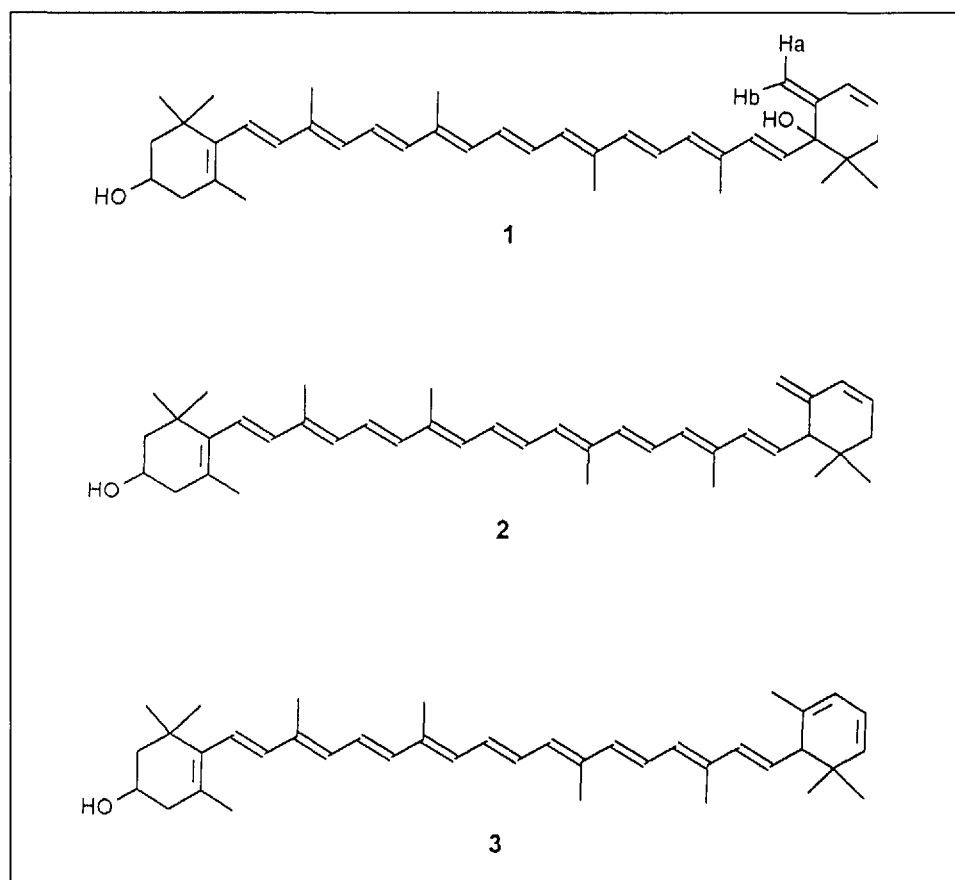
nance appearing at higher field to H_b (NOE $H_b \leftrightarrow$ H–C(7')). Analogously the *singlets* at 5.03 and 5.00 ppm in the spectrum of nigroxanthin (1) were assigned to H_a and H_b.

The resonances for $H_3C(16')$ and $H_3C(17')$ can be found in the expected spectral region, but, as in the case of 2, their assignments may be exchanged. The signal for HO–C(6') was not identified.

The ^{13}C resonances of C(1'), C(2'), C(5'), and C(6') can be identified directly from their characteristic δ values and informations out of the DEPT-135 experiment, while line assignment for C(3'), C(4'), C(16'), and C(17') is not feasible without any doubt. C(18') gives a resonance at 105.09 ppm, typically for an exocyclic CH_2-sp^2-C-nucleus. It must be mentioned that its relative signal intensity is unexpectedly weak; reasons for this observation are unknown.

Applications of H,H-COSY, C,H-shift correlation and COLOC experiments to 2 and 3 allow extensive line assignments for ^1H and ^{13}C resonances. Based on comparison with these data sets, most of the ^1H and a number of the ^{13}C signals of the nigroxanthin olefinic chain can be assigned. Inspection of the ^nJ(H,H) values confirms the supposed (all-*E*)-configuration of 1.

Based on the spectroscopic data, especially the NMR investigations, nigroxan-

thin (1) was identified as (all-*E*)-3',4'-didehydro-β,γ-carotene-3,6'-diol. The configuration at C(3) and C(6') remains at the moment unknown. The total synthesis of optically active compounds with the constitution of 1 is in progress.

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* C_{18}, 6 μm, endcapped. Mobile phase: eluent *A* 12% H_2O in MeOH, eluent *B*: MeOH, eluent *C*: acetone/MeOH 1:1. Gradient program: 0–2 min: 100% *A*; –10 min: to 80% *A*/ 20% *B*; –18 min: to 50% *A*/50% *B*; –25 min; to 100% *B*; –27 min: 100% *B*, –34 min: to 100% *C*: –41 min 100% *C* (linear steps). UV/VIS: *Beckman DU-65*. CD: *Jobin-Yvon Dichrograph-6* in MeOH at r.t. NMR: *Varian Unity 300* (^1H: 300 MHz, ^{13}C: 75.43 MHz), *Bruker AM 400* (^1H: 400.14 MHz, ^{13}C 100.61 MHz), *Bruker AC 300* (^1H: 300.13 MHz, ^{13}C 75.47 MHz) 0.2 mg of 1, 11 mg of 2, 6 mg of 3 in 0.5 ml of $CDCl_3$ at 20°. Chemical shifts (δ) in ppm (relative to the solvent signal), coupling constants (*J*) in Hz. MS: *Jeol JMS-01-SG-2*.

Isolation. A detailed description of the general isolation procedure has been given in [3]. After desorption of zone 7 [3], nigroxanthin (1) was precipitated in benzene/hexane (12 mg, m.p. 99°, purity 86%). After recrystallization (benzene/ hexane) the product was submitted to CC: 3 columns 6 x 30 cm, $CaCO_3$ *(Biogal,* Hungary), 3– 4% acetone in hexane. Picture after development:

30 mm yellow: unknown; 60 mm intermediate zone; 30 mm yellow (nigroxanthin (1)); 8 mm rose-colored: unknown, 10 mm intermediate zone, 5 mm yellow: unknown, 20 mm intermediate zone, 20 mm: pale yellow. After the development the column was extruded and cut into pieces. After desorption 1 was crystallized (benzene/ hexane) to give 8 mg of red crystals (m.p. 125– 127°, purity > 98%. DC: R_f 0.64 (silica F_{254} *(Merck 5554)*, benzene/AcOEt/MeOH 7:2:1).

Spectroscopical Data of 1. IR (KBr): 883*s*, 3425*m*. UV/VIS (benzene): 487 (1.157), 457 (1.293), 434 (0.880). UV (hexane): 472, 441, 421. UV (Et$_2$O): 473, 445. UV (MeOH): 472, 444. UV (EtOH): 474, 447. MS: 566 (100, *M$^+$*), 548 (11), 530 (2), 474 (9), 119 (28), 105 (22). ^1H- and ^{13}C-NMR: *cf.* the *Table*.

Nigroxanthin monoacetate. Acetylation according to [9]. Crystallization: benzene/MeOH; m.p. 82–84°. DC: R_f 0.87 (silica gel F_{254} *(Merck 5554)*, benzene/AcOEt/MeOH 7:2:1). IR (KBr): 3420*m*. UV/VIS (benzene): 487, 457, 433. EI-MS: 608 (100, *M$^+$*, $C_{42}H_{56}O_3$), 548 (5), 516 (15), 502 (3), 456 (4).

Nigroxanthin Trimethylsilyl Ether. Silylation according to [10] DC: R_f 0.85 (silica gel F_{254}, *(Merck 5554)*, benzene/AcOEt 7:3). IR (KBr): 895*s*, 1715*s*, 3470*m*. UV/VIS (benzene): 487, 457, 433. EI-MS: 638 (35, *M$^+$*, $C_{43}H_{62}O_2Si$), 566 (100), 548 (4), 546 (15), 368 (12).

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