Nigrosoxanthin (3',4'-Didehydro-\(\beta\),\(\gamma\)-carotene-3,6'-diol), a New Carotenoid Isolated from Paprika (Capsicum annuum var. longum nigrum)

József Dél(*), Zoltán Matus(*), Péter Molnár(*), Gyula Tóth(*), Gábor Szalontai(*), Andrea Steck(1), and Hanspeter Pfander(*)

Abstract. From red paprika (Capsicum annuum var. longum nigrum) nigrosoxanthin (1) was isolated as a minor carotenoid and, based on its spectral data, identified as (all-\(E\))-3',4'-didehydro-\(\beta\),\(\gamma\)-carotene-3,6'-dicol.

Introduction

The different varieties of paprika (Capsicum annuum) have been investigated for a long time. It has been established that capsanthin ((3R,3'S,5'R)-3,3'-dihydroxy-\(\beta\),\(\kappa\)-carotene-6'-one) and capsorubin ((3S,5R,3'S,5'R)-3,3'-dihydroxy-\(\kappa\)-carotene-6,6'-dione), both of which contain the five membered ring \(\kappa\)-end group, are the most abundant carotenoids in these vegetables [1]. Furthermore, many other carotenoids with interesting structures, especially those with the oxabicyclo-\(\beta\)-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 the biosynthesis of the \(\kappa\)-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 cyclovioxanthin [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 CaCO\(_3\) column (Biogal, Hungary) was further investigated. From this zone, a new carotenoid, for which the name 'Nigrosoxanthin' (1) is proposed, was isolated and crystallized from benzene/hexane (m.p. 125–127\(^\circ\)).

The UV/VIS spectrum (\(\lambda_{\text{max}}\) 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 LiAlH\(_4\), or HCl/ArO\(_2\) indicating that no carbonyl or 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\(_2\)O]\(^+\)), 530 ([M – 2H\(_2\)O]\(^+\)), 474 ([M – 2\(\text{CH}_3\)OH]\(^+\)), 119, and 105. Characteristic signals for allenes, acetyl, carbonyl, 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\(_3\))\(_2\)SiCl/(CH\(_3\))\(_2\)Si\(_2\) resulted in a mono-trimethylsilyl ether (MS: 638 (\(M^+\))). These derivatives give an indication that nigrosoxanthin (1) contains one \(\text{prim.}\) or \(\text{sec.}\) and one tert. O\(_\text{H}\) 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 \(^1\text{H}\)- and \(^13\text{C}\)-NMR spectra of nigrosoxanthin (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 nigrosoxanthin.

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 \(\nu(H,H)\) values are listed. The \(^1\text{H}\) and \(^13\text{C}\) resonances of the well-known \(\beta\)-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 \(^1\text{H}\)-NMR resonances for the geminal protons H\(_{2}\)C(2') in the \(\gamma\)-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 H\(_2\)C(2') and H\(_2\)C(3') as well as between H\(_2\)C(3') and H\(_2\)C(4') are visible, so that the signals for both olefinic protons can be assigned. Two slightly broadened singlets, with \(\delta\) values (5.03 and 5.00 ppm) typical for exocyclic olefinic CH\(_2\) protons, correspond to the nuclei H\(_2\)C(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 2D NOE difference experiments that the signal appearing at lower field corresponds to H\(_a\) (NOE H\(_a\) ↔ H–C(4')), and the reso-
Table: $^1$H- and $^{13}$C-NMR Data for 1, 2, and 3

| C  | $^1$H  | $^1$C  |
|----|-------|-------|
|    | 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 |
| 3  | 4.00, OH: 1.56 | 4.00, OH: 1.48 | 4.00, OH: 1.40 |
| 4  | 2.39 eq. 2.05 ax | 2.39 eq. 2.02 ax | 2.39 eq. 2.04 ax |
| 5  | 126.16 | 126.16 | 126.15 |
| 6  | 137.77 | 137.77 | 137.77 |
| 7  | 6.10 (AB) | 6.10 (AB) | 6.11 (AB) |
| 8  | 6.15 (AB) | 6.15 (AB) | 6.15 (AB) |
| 9  | 135.57 | 135.47 | 135.63 |
| 10 | 6.19 (10.4) | 6.17 (12.2) | 6.16 (12.0) |
| 11 | 6.65 (10.4, 14.8) | 6.64 (12.2, 14.9) | 6.63 (12.0, 14.9) |
| 12 | 6.36 (14.8) | 6.36 (14.9) | 6.36 (14.9) |
| 13 | 136.44 | 136.39 | 136.37 |
| 14 | 132.62 | 132.62 | 132.62 |
| 15 | 130.10 | 130.11 | 130.11 |
| 16 | 28.73 | 28.73 | 28.72 |
| 17 | 30.26 | 30.26 | 30.26 |
| 18 | 21.82 | 21.61 | 21.62 |
| 19 | 12.82 | 12.81 | 12.81 |
| 20 | 12.82 | 12.81 | 12.81 |
| 1' | 49.81 | 33.45 | 34.81 |
| 2' | 1.94, 2.01 (AB) | 5.32 (9.4) | 29.70 |
| 3' | 5.75 (9.4, 5.1) | 5.75 (9.4, 5.1) | 127.81 |
| 4' | 5.60 (5.1) | 5.60 (5.1) | 127.81 |
| 5' | 149.32 | 145.68 | 137.22 |
| 6' | 73.67 | 55.49 | 55.72 |
| 7' | 5.57 (15.1, 10.0) | 5.57 (15.1, 10.0) | 128.00 |
| 8' | 6.12 (15.5) | 6.12 (15.5) | 128.00 |
| 9' | 135.64 | 135.64 | 135.64 |
| 10' | 6.16 (11.9) | 6.15 (11.7) | 130.66 |
| 11' | 6.60 (11.7, 15.0) | 6.60 (11.7, 15.0) | 125.01 |
| 12' | 6.33 (15.0) | 137.28 | 137.14 |
| 13' | 136.47 | 136.47 | 136.53 |
| 14' | 6.24 | 6.25 | 132.35 |
| 15' | 6.64 | 6.64 | 132.35 |
| 16' | 0.88 | 0.93 | 25.33 |
| 17' | 0.90 | 0.90 | 25.33 |
| 18' | 4.88 H, 5.00 H | 4.88 H, 4.81 H | 129.92 |
| 19' | 1.92 | 1.92 | 129.92 |
| 20' | 1.96 | 1.96 | 129.92 |

Chemical shift values: $\delta$ [ppm]. $J$(H,H) coupling constant values: $\langle J$ [Hz]).

\(\text{a)–d) Assignment may be interchanged.}\)
\(\text{e) Not assigned.}\)
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