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'Prenigroxanthin' [(all-*E*,3*R*,3′*S*,6′*S*)-β,γ-carotene-3,3′,6′-triol], a novel carotenoid from red paprika (*Capsicum annuum*)

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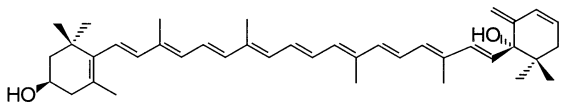
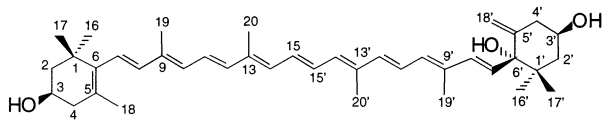
Abstract—From the ripe fruits of red paprika (*Capsicum annuum*) prenigroxanthin, a minor carotenoid was isolated and, based on the spectral data and the proposed biosynthesis, identified as (all-*E*,3*R*,3′*S*,6′*S*)-β,γ-carotene-3,3′,6′-triol.

During our investigations of different species of paprika (*Capsicum annuum*), some novel carotenoids such as cycloviolaxanthin [(3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*R*)-3,6,3′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,5′-diol], cucurbitaxanthin A [(3*S*,5*R*,6*R*,3′*R*)-3,6-epoxy-5,6-dihydro-β,β-carotene-5,3′-diol] and B [(3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-3,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol] and capsanthin 3,6-epoxide [(3*S*,5*R*,6*R*,3′*S*,5′*R*)-3,6-epoxy-5,6-dihydro-5,3′-dihydroxy-β,κ-caroten-6′-one], all containing the 7-oxabicyclo[2.2.1]heptyl end group have been isolated and characterized.[1,2] We have also published the isolation of 5,6-diepikarpoxanthin [(3*S*,5*S*,6*S*,3′*R*)-5,6-dihydro-β,β-carotene-3,5,6,3′-tetrol], 5,6-diepilatoxanthin [(3*S*,5*S*,6*S*,3′*S*,5′*R*,6′*S*)-5′,6′-epoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,6,3′-tetrol], and 5,6-diepicapsokarpoxanthin [(3*S*,5*S*,6*S*,3′*S*,5′*R*)-5,6-dihydro-3,5,6,3′-tetrahydroxy-β,κ-caroten-6′-one], which all possess the (3*S*,5*S*,6*S*)-trihydroxy-β-end group, and of 6-epikarpoxanthin [(3*S*,5*R*,6*S*,3′*R*)-5,6-dihydro-β,β-carotene-3,5,6,3′-tetrol] containing the (3*S*,5*R*,6*S*)-trihydroxy-β-end group, from red paprika.[3] In a previous paper,[4] we described the isolation and structure elucidation of nigroxanthin (3′,4′-didehydro-β,γ-carotene-3,6′-diol) (**1**) containing the 6-hydroxy-γ-end group, but the assignment of the configuration at C(6′) remained unknown. These compounds may be formed from antheraxanthin [(3*S*,5*R*,6*S*,3′*R*)-5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol] and violaxanthin [(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol], and their occurrence may be interrelated with the biosynthesis of the κ-end group, which has not been clarified in every detail yet.

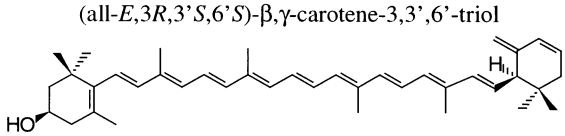
In this paper we report on the isolation and characterization of a new carotenoid (**2**), for which the name 'prenigroxanthin' is proposed, from red spice paprika (*Capsicum annuum*, var. *longum*).



Nigroxanthin (**1**)
(all-*E*,3*R*,6′*S*)-3′,4′-Didehydro-β,γ-carotene-3,6′-diol



Prenigroxanthin (**2**)
(all-*E*,3*R*,3′*S*,6′*S*)-β,γ-carotene-3,3′,6′-triol



Desoxylutein II (**3**)
(all-*E*,3*R*,6′*R*)-3′,4′-Didehydro-β,γ-carotene-3-ol

Scheme 1.

Keywords: carotenoids; isolation; structure elucidation; paprika; *Capsicum annuum*.

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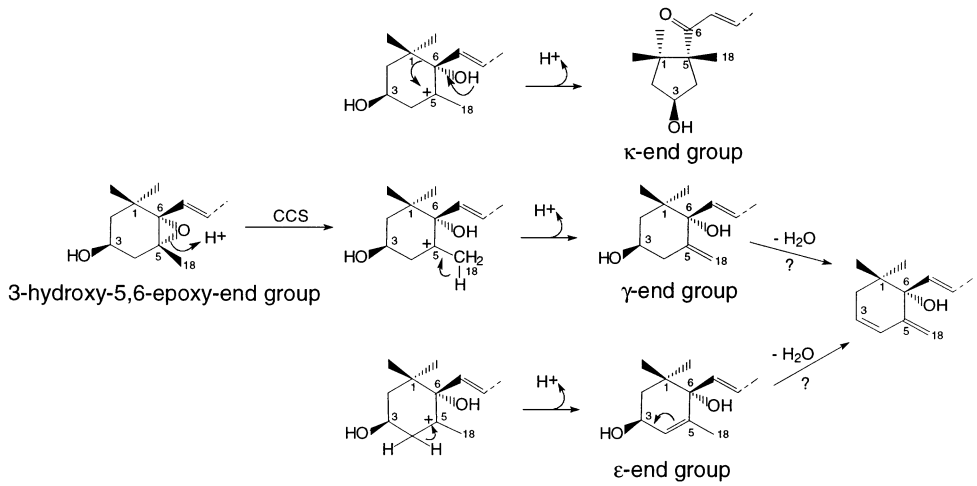

Scheme 2. Possible formation of different end groups from the 3-hydroxy-5,6-epoxides after Camara.[8]

Eight kilos of red paprika pods were first extracted with MeOH, then with Et_2O. After saponification, the carotenoids from the methanolic fraction were precipitated and separated by column chromatography on $CaCO_3$ (benzene/hexane).

Repeated column chromatography yielded 17 mg of 5,6-diepikarpoxanthin, 1 mg of 6-epikarpoxanthin, 0.5 mg of 5,6-diepilatoxanthin, 30 mg of capsorubin [(3*S*,5*R*,3′*S*,5′*R*)-3,3′-dihydroxy-κ,κ-carotene-6,6′-dione], 508 mg of capsanthin [(3*R*,3′*S*,5′*R*)-3,3′-dihydroxy-β,κ-caroten-6′-one], and 1 mg of prenigroxanthin (**2**) (mp 154–158°C), respectively.

The structure of compound **2** was determined by its UV–vis, CD, NMR (^1H, ^1H–^1H COSY, T ROESY) and mass spectra (Scheme 1).

The UV–vis spectrum (λ_{max}, benzene: 487, 457, 434 nm, no *cis*-peak) showed that the compound contains an (all-*E*)-decaene chromophore. With $NaBH_4$ or HCl/AcOH no reaction took place, indicating that no carbonyl or 5,6-epoxy groups are present. The EI-MS exhibited the signal for the molecular ion at m/z 584 (100, M^+), which corresponds to $C_{40}H_{56}O_3$.

For full characterization of prenigroxanthin (**2**) the NMR data were compared with those of nigroxanthin (**1**), isolated earlier from the red paprika, and of desoxylutein II (**3**).[4] ^1H NMR, ^1H–^1H COSY and T ROESY experiments allowed complete ^1H signal assignments.[5] Due to decomposition under the measuring conditions, no ^{13}C NMR data were obtained. The δ(H) and $J_{H,H}$ values of the 3-hydroxy-β end group are identical with the corresponding data from the literature.[6] In the γ-end group, the axial H-C(2′) and axial H-C(4′) can be assigned by their ROESY signal. The coupling constant between the axial H-C(4′) and the H-C(3′) of 9.3 Hz and the axial H-C(2′) and the H-C(3′) of 10.2 Hz indicate that HO-C(3′) is equatorial. Two singlets at 4.97 and 4.85 ppm typical for exocyclic olefinic CH_2 protons correspond to the nuclei $H_2C(18')$ which may be arbitrarily named H_a and H_b.

Prenigroxanthin (**2**) exhibited a conservative CD spectra, which confirms the (3*R*)- and (3′*S*)-configuration, but does not give any indication for the configuration at C(6′).[7]

As the configuration at C(6′) of **1** and **2** has not yet been clarified by modern spectroscopic methods, the biosynthetic pathway of paprika carotenoids was taken into account.

Recently, the capsanthin–capsorubin synthase (CCS), an enzyme catalyzing the conversion of 5,6-epoxy-end groups into κ-end groups was isolated and characterized,[8] and certain similarities with the *C. annuum* lycopene cyclase, the enzyme catalyzing the cyclization of lycopene, were observed.[9] The fact that CCS also exhibits lycopene cyclase activity is likely to be related to similarities in the chemical mechanisms leading to the formation of β-rings, as in β,β-carotene, and of κ-rings, as in capsanthin and capsorubin. In both mechanisms, a carbenium ion at C(5) is formed as an intermediate. On the basis of the above described reaction mechanism, we have suggested a new mechanism for the formation of 3,5,6-trihydroxy-carotenoids isolated from red paprika.[3] During the enzyme catalyzed hydrolysis of 5,6-epoxy-carotenoids, the configuration at C(5) may change, but remains unchanged at C(6). Based on this biochemical aspect, we suggest the (6′*S*)-configuration for both *nigroxanthin* (**1**) and *prenigroxanthin* (**2**) (Scheme 2).

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5. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.08 (s, 6H, CH_3(16,17)), 1.16 (s, 3H, CH_3(16′)), 1.31 (s, 3H, CH_3(17′)), 1.49 (ψt, J=12.4 Hz, 1H, H-2$_{ax}$), 1.74 (s, 3H, CH_3(18)), 1.78 (ddd, J=12.1, 3.2, 2.1 Hz, 1H, H-2$_{eq}$), 1.84 (dd, J=13.5, 10.2 Hz, 1H, H-2′$_{ax}$), 1.96 (dd, J=13.5, 4.7 Hz, 1H, H-2′$_{eq}$), 1.99 (s, 12H, CH_3(19,20,19′,20′)), 2.05 (dd, J=15.7, 9.7 Hz, 1H, H-4$_{eq}$), 2.38 (ddd, J=15.7, 6.2, 3.2 Hz, 1H, H-4$_{ax}$), 2.40 (dd, J=13.1, 9.3, 1H, H-4′$_{ax}$), 2.64 (ddd, J=13.1, 5.0, 1.5 Hz, 1H, H-4′$_{eq}$), 4.0 (m, 1H, H-3), 4.22 (m, 1H, H-3′), 4.85 (s, 1H, H$_b$-18′), 4.97 (s, 1H, H$_a$-18′), 6.10 (d, J=18.7 Hz, 1H, H-7), 6.15 (d, J=15.6 Hz, 1H, H-7′), 6.16 (d, J=18.7 Hz, 1H, H-8), 6.16 (d, J=11.5 Hz, 1H, H-10), 6.19 (d, J=11.5 Hz, 1H, H-10′), 6.26 (d, J=15.6 Hz, 1H, H-8′), 6.26 (AB spin system, 1H, H-14), 6.27 (AB spin system, 1H, H-14′), 6.36 (d, J=15.0 Hz, 2H, H-12,12′), 6.62 (AB spin system, 1H, H-15′), 6.64 (dd, J=15.0, 11.5 Hz, 2H, H-11,11′), 6.64 (AB spin system, 1H, H-15).
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7. CD (EPA, rt): 212 (−3.35), 241 (+1.07), 273 (−2.34), 329 (+0.60); CD (EPA, −180°C): 204 (−3.40), 235 (+4.06), 279 (−9.56), 303 (−0.63), 312 (−0.28).
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