Carotenoids of Rhizobia. III. 2′,3′-*trans*-Dihydroxy-2-nor-β, β-carotene-3,4-dione, a Novel Carotenoid from a Mutant of *Rhizobium lupini*

Peter Beyer, Hans Kleinig,

Institut für Biologie II, Lehrstuhl für Zellbiologie, Schänzlestraße 1, D-7800 Freiburg

Walter Meister, and Gerhard Englert

Central Research Units, F. Hoffmann-La Roche & Co., Ltd., Basel

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Nor-Carotenoid, *Rhizobium lupini*

The mutant strain 1-289 of *Rhizobium lupini* containes in addition to the normal carotenoid pattern a violet carotenoid whose structure was derived by MS and 270 ^{1}H-NMR as 2′,3′-*trans*-dihydroxy-2-nor-β,β-carotene-3,4-dione. The possible biochemical origin of this compound is discussed.

Introduction

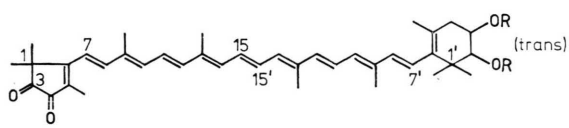
The soil and root nodule bacterium *Rhizobium lupini* has been shown recently to contain highly substituted β,β-carotene derivatives (main pigment: 2,3,2′,3′-di-*trans*-tetrahydroxy-β,β-carotene-4-one and 2,3,2′,3′-di-*trans*-tetrahydroxy-β,β-carotene) [1]. Colonies of the mutant strain 1-289 exhibited a deeper red color on agar plates than did the parent strain. Chromatography of a pigment extract from this mutant revealed one additional violet pigment the structure determination of which will be described in the present publication.

Results

The violet pigment of the mutant accounted for about 3% of the total carotenoid content. Its absorption spectrum exhibited a broad maximum at 510 nm (ethanol). Upon reduction with $NaBH_4$ a spectrum with maxima at 458 and 482 and an inflexion at 434 nm (ethanol) was obtained. These absorption characteristics pointed to the presence of one five-membered ring of roserythrin type in the molecule [2].

The native violet pigment was less polar than the tetrahydroxy- and trihydroxy-β,β-carotene derivatives occurring as the main pigments. An indication for the presence of two free hydroxyl groups in the molecule was obtained by acetylation and subsequent thin layer chromatography. The reduced compound ($NaBH_4$) was believed to contain four rather than three hydroxyl groups as judged by its polarity on thin layer chromatography.

The final structure I of the native pigment was unequivocally derived from MS and 270 MHz ^{1}H-NMR data of the peracetylated derivative **I a**.



I. Native pigment R=H

Ia. Peracetylated R= $COCH_3$

Scheme: Chemical structures.

The mass spectrum of **I a** showed the molecular ion at *m/e* 666. The acetoxy groups are consecutively eliminated as acetic acid to give fragment peaks at *m/e* 606 and 546. The typical fragmentation of carotenoids, namely the elimination of toluene and xylene, is only weak here (M-92 6%; M-106 5%) in accordance with the spectrum of violerythrin [3] (2,2′-dinor-β,β-carotene-3,4,3′,4′-tetrone). Both the molecular ion and the fragment peaks were accompanied by ($M-H_2$)-peaks probably originating from artifacts.

The 270 MHz ^{1}H-NMR spectrum (see experimental part) revealed that the molecule must be built up from two unsymmetrical halves. Thus, one part of the spectrum agreed very closely with the spectrum of 2,3,2′,3′-di-*trans*-tetrahydroxy-β,β-carotene [1], while the other part was found to be

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practically identical with the spectrum of violerythrin [3, 4], the total synethesis of which was reported very recently [5]. Hence, structure **I a** is proposed for the peracetylated pigment **I**.

Discussion

Nor-carotenoids with five-membered rings have rarely been found in nature. Examples are actinioerythrin (3,3′-dihydroxy-2,2′-dinor-β,β-carotene-4,4′-dione-3,3′-diacylate) [3] and 2-nor-astaxanthin diester (3,3′-dihydroxy-2-nor-β,β,carotene-4,4′-dione-3,3′-diacylate) [2] from *Actinia equina*. Upon alkali treatment these structures are converted into violerythrin and roserythrin, respectively, containing cyclopentenedione end groups which have been claimed not to occur naturally.

The nor-carotenoid described in the present investigation seems not to be an artifact caused during extraction or separation. Alkaline conditions were avoided and parallel growth and extraction, including extraction using acidified acetone, of both the parent strain and the mutant always gave the same differences in the pigment patterns. Furthermore, the parent strain does not contain nor-carotenoids and carotenoid ester. The mutant also lacks nor-carotenoids other than that described. Growth colonies of the mutant, on the other hand, are more deeply colored than colonies of the parent strain.

Hertzberg and Liaaen-Jensen [6] postulated a biosynthetic sequence of actinioerythrin from astaxanthin via a benzylic acid rearrangement. Later, the oxidation of diosphenol to cyclopentenediones was also achieved *in vitro* using MnO_2 [7]. Thus it is very likely that the nor-carotenoid described is a derivative of 2,3,2′,3′-di-*trans*-tetrahydroxy-β,β-carotene-4-one, which seems to be very suited for such a rearrangement due to its substitution at C(2). We conclude, however, that the nor-carotenoid described is not an artifact produced *in vitro* but originates *in vivo* from 2,3,2′,3′-di-*trans*-tetrahydroxy-β,β-carotene-4-one by an unknown predisposition of the mutant.

Experimental

Materials and Methods

Rhizobium lupini 1-289 was obtained from Prof. W. Heumann, Erlangen. Bacteria were grown in 0.8% nutrient broth (Merck) in 100 ml batches in 500 ml Erlenmayer flasks on a shaker.

Isolation of pigments, chemical methods used and instrumentation were as previously described [1].

Spectroscopic data

2′,3′-*trans*-dihydroxy-2-nor-β,β-carotene-3,4-dione (**I**), pigment yield about 0.6 mg. Visible light (ethanol): 510 nm; reduced from, 434, 458, 482 nm.

MS of peracetylated form (**I a**): *m/e* 666 (100, M); 664 (28); 606 (28); 604 (20); 574 (6); 560 (5); 546 (30).

^{1}H-NMR of **I a** in $CDCl_3$:

1.003 and 1.085 ppm (s, 3H each, gem. methyl groups at C(1)); 1.423 (s, 6H, gem. methyl groups at C(1′)); 1.707 (s, 3H, methyl group at C(5′)); 1.98 and ca. 2.00 (methyl groups at C(9′) and C(13′)); ca. 2.00 (methyl at C(13)); ca. 2.08 (methyl groups at C(5) and C(9)); ca. 2.04 and 2.10 (2 s, O-acetyl at C(2′) and C(3′)); 5.04 (*d*, $J = 11$ Hz, H at C(2′)); ca. 5.14 (m, H at C(3′)); 6.04 (*d*, $J \sim 16$ Hz, 1H, H at C(7′)); 6.12 (*d*, $J \sim 16$ Hz, 1H, H at C(8′)); 6.18 (*d*, $J \sim 12$ Hz, 1H, H at C(10′)); 6.32 (*d*, $J \sim 11$ Hz, 1H, H at C(14′)); 6.39 (*d*, $J \sim 15$ Hz, H at C(12′)); 6.40 (*d*, $J \sim 11$ Hz, H at C(14)); 7.17 (*d*, $J \sim 16$ Hz, H at C(7) or C(8)); 6.53 to 6.75 (m, ca. 8H, remaining protons).

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