260. Carotenoids of Rhizobia. IV. Isolation and Structure Elucidation of the Carotenoids of a Mutant of *Rhizobium lupini*

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Summary

The structures of the main carotenoid pigments from the mutant 1-207 of *Rhizobium lupini* were elucidated by spectroscopic techniques (UV./VIS., CD., 270 MHz ^{1}H-NMR., and MS.). Ten carotenoids were identified, namely β,β-carotene (**1**), β,β-caroten-4-one (echinenone, **2**), β,β-carotene-4,4′-dione (canthaxanthin, **3**), (3*S*)-3-hydroxy-β,β-caroten-4-one ((3*S*)-3-hydroxyechinenone, **4**), (2*R*,3*R*)-β,β-carotene-2,3-diol (**5**), (3*S*)-3-hydroxy-β,β-carotene-4,4′-dione ((3*S*)-adonirubin, **6**), (2*R*,3*S*)-2,3-dihydroxy-β,β-caroten-4-one (**7**), (2*R*,3*S*)-2,3-dihydroxy-β,β-caroten-4,4′-dione (**8**), (2*R*,3*S*,2′*R*,3′*R*)-2,3,2′,3′-tetrahydroxy-β,β-caroten-4-one (**9**) and the corresponding (2*R*,3*S*,2′*R*,3′*S*)-4,4′-dione (**10**). Structures **5**, **7**, **8** and **10** have not been reported before. From the observed carotenoid pattern it is concluded that in this mutant the oxidation to 4-oxo compounds is favoured compared to the hydroxylation at C(3) and C(2).

Introduction. – The carotenoid pigments of the soil and root nodule bacterium *Rhizobium lupini* strain 1-250 (parent strain) are characterized by highly substituted β,β-carotenes with unusual hydroxylations at the C(2,2′) positions. The structure of the main pigments (with relative geometry only) is 2,3,2′,3′-di-*trans*-tetrahydroxy-β,β-caroten-4-one and β,β-carotene-2,3,2′,3′-di-*trans*-tetrol [1]. The absolute configuration of these carotenoids is now given as in **9** and **11** (see *Table* and *Scheme*).

A 'brownish' coloured mutant 1-207 of this strain was obtained [2] which revealed a much more complex carotenoid pattern than the parent strain. The carotenoids of this mutant which include several new structures are the subject of the present investigation.

Results. – Based on the UV./VIS., ^{1}H-NMR. and mass spectroscopic results, the elucidation of the (relative) structures of the different components **1**–**10** was straightforward. The 270 MHz ^{1}H-NMR. spectra were particularly easily interpreted since they could be compared directly to and additively built up from the corresponding spectra of the symmetric compounds, *e.g.* β,β-carotene (**1**), canthaxanthin (**3**), astaxanthin diacetate *etc.* [3] [4]. In fact, a full assignment of even the strongly overlapping olefinic part of the spectra was in most cases possible in this

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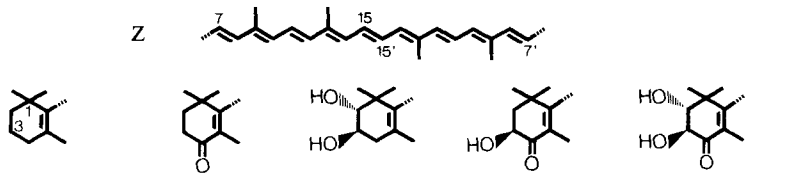
Table. *Carotenoids of mutant 1-207 of* Rhizobium lupini *listed with increasing polarity.*

		Compounds	% of total
1	aza	β,β-carotene	8
2	bza	β,β-caroten-4-one	6
3	bzb	β,β-carotene-4,4'-dione	4
4	dza	(3*S*)-3-hydroxy-β,β-caroten-4-one	2
5	cza	(2*R*,3*R*)-β,β-carotene-2,3-diol	1
6	dzb	(3*S*)-3-hydroxy-β,β-carotene-4,4'-dione	1
7	eza	(2*R*,3*S*)-2,3-dihydroxy-β,β-caroten-4-one	6
8	ezb	(2*R*,3*S*)-2,3-dihydroxy-β,β-carotene-4,4'-dione	8
9	ezc	(2*R*,3*S*,2'*R*,3'*R*)-2,3,2',3'-tetrahydroxy-β,β-caroten-4-one	4
10	eze	(2*R*,3*S*,2'*R*,3'*S*)-2,3,2',3'-tetrahydroxy-β,β-carotene-4,4'-dione	52
		Unknowns	10

Scheme



a b c (2*R*,3*R*) d (3*S*) e (2*R*,3*S*)

11 czc = pigment **2** of [1]

way and this led to an unequivocal derivation of the structures. In all cases, the conjugated polyene chain was found to be all-*trans*.

In most cases sufficient material was available to obtain qualitative CD. spectra of the chiral compounds thus also enabling an assignment of the absolute configuration of their optical centers to be made. In the other cases, the absolute configuration is assumed to be the same by analogy as will be discussed below.

The quantitative carotenoid distribution of the mutant 1-207 is shown in the *Table*. Ten structures could be positively identified four of which have, to our knowledge, not yet been described. In addition, several further carotenoids (about 10% of the total carotenoid content) which are presumably trihydroxy-oxo compounds could not be separated in sufficient purity for ^{1}H-NMR. and MS. investigation.

β,β-Carotene (**1**); *β,β-caroten-4-one* (echinenone **2**); *β,β-carotene-4,4'-dione* (canthaxanthin **3**). These compounds were identified from their UV.-spectral and chromatographic properties and by MS. (**2** and **3**, see experimental part).

(3S)-3-Hydroxy-β,β-caroten-4-one (hydroxyechinenone **4**). This pigment showed an echinenone-type chromophore. The presence of one hydroxyl group was inferred from an acetylation test. Saponification of the acetate led to the formation of the corresponding diosphenol which indicated the position of the hydroxyl group at C(3). The MS. of the acetylated pigment **4** shows the molecular ion at *m/z* 608 and fragment ions at 566 (elimination of ketene) and 548 (loss of acetic acid).

The 270 MHz ^{1}H-NMR. spectrum of acetylated **4** closely corresponds to a superposition of the spectra of astaxanthin diacetate [3] and β,β-carotene (**1**) [4].

The presence of the end-group of the former is revealed by its signals at 1.229 and 1.351 (3 H each, CH_3–C(1)); 1.907 (3 H, CH_3–C(5)); 2.193 (3 H, OAc) and 5.526 ($d \times d$, $J = 13$ and 6.5 Hz, 1 H, H_{ax}–C(3)). The β end-group is identified by its signals at 1.030 (6 H, CH_3–C(1')) and 1.723 (3 H, CH_3–C(5')). All these signals and also those from the olefinic part are found as in the spectrum of a synthetic sample [5] [6] unequivocally proving the structure.

The CD. spectrum of acetylated **4** closely corresponds in shape and sign to the recently published CD. spectrum of synthetic (3*S*)-3-hydroxyechinone [5] thus proving the (3*S*)-configuration of **4**. The negative maximum at 366 nm, where the synthetic compound shows a positive one, is probably due to a small portion of *cis* isomers or decomposition products formed during the measurement.

*(2*R*, 3*R*)-β,β-Carotene-2, 3-diol* (**5**). This compound, showing a typical β,β-carotene absorption spectrum, was identified as a dihydroxy compound by polarity and acetylation tests. The pigment did not give an ether when mixed with acidified ethanol which excluded the position of an allylic hydroxyl group at C(4, 4').

The molecular ion at *m/z* 652 in the MS. of acetylated **5** is very intense. Owing to the presence of impurities the typical carotenoid fragmentation, namely the loss of toluene from the molecular ion, is not clearly seen. However, at an ionization energy of 12 eV a corresponding peak at *m/z* 560 with *ca.* 12% relative intensity is detected.

The ^{1}H-NMR. spectrum of the diacetate of **5** clearly reflects the presence of a 2,3-*trans*-dihydroxy-β end-group as was previously described [1] and, in addition, all relevant signals for the unsubstituted β end-group as given above for compound **4** (see also experimental part). The CD. spectrum could not be obtained due to lack of material. However, in analogy to pigments **9** and **11** we assume a (2*R*,3*R*) configuration (see below). This structure has not been found hitherto in nature [7].

*(3*S*)-3-Hydroxy-β,β-carotene-4, 4'-dione* (adonirubin, phoenicoxanthin, **6**, see [7]). This compound exhibits a canthaxanthin-type chromophore indicating the presence of two keto groups at C(4) and C(4') in conjugation with the polyene chain. An additional hydroxyl group was assigned to position C(3) by the criteria used above for pigment **4**. The MS. of acetylated **6** is very similar to that of acetylated **4** with all relevant peaks shifted upward by 14 mass units due to the presence of a further keto-function (see exp. part).

The ^{1}H-NMR. spectrum of the acetate of **6** showed all relevant signals of the end-groups of astaxanthin diacetate and canthaxanthin. Its spectrum was identical to that of a synthetic sample [5] [6].

The shape and positions of the CD. maxima of the acetate of **6** are identical to those of (3*S*)-astaxanthin and its diacetate [3] thus establishing the (3*S*) configuration.

*(2*R*, 3*S*)-2, 3-Dihydroxy-β,β-caroten-4-one* (**7**). The echinenone-type absorption spectrum and the alkali lability of this pigment pointed to the presence of the typical 2,3-dihydroxy-4-keto end-group of *Rhizobium* carotenoids [1]. The reduced polarity, however, when compared with the known tetrahydroxy-keto and trihydroxy-keto structures [1] led to the conclusion that the second β end-group was unsubstituted.

The MS. of acetylated **7** shows the molecular ion at *m/z* 666. The acetates are lost by the elimination of ketene and acetic acid forming the fragments *m/z* 606 and 564. In the middle of the spectrum the typical fragments *m/z* 245 and 203 indicate the presence of an astacene-type end-group [8] formed from the original one by the elimination of acetic acid.

Although the ^{1}H-NMR. spectrum of the diacetate of **7** indicated the presence of some impurities, the existence of the 2,3-*trans*-diacetoxy-4-keto-*β* end-group is revealed by all characteristic signals as described previously [1]: 1.147 and 1.319 (CH_3–C(1)); 1.914 (CH_3–C(5)); 2.124 and 2.169 ($OCOCH_3$); 5.325 and 5.533 (*AB*, $J_{2,3}$= 11.8 Hz, H_{ax} at C(2) and C(3)). The presence of the unsubstituted *β* end-group is again evidenced by signals at 1.030 (CH_3–C(1′)) and 1.722 (CH_3–C(5′)) (see also exper. part).

No CD. spectrum of this compound was measured but it seems reasonable to assume the same absolute configuration (2*R*,3*S*) as in the symmetric pigment **10** (see below). This structure has not been described so far [7].

*(2*R,3S*)-2,3-Dihydroxy-β,β-carotene-4,4′-dione* (**8**). This pigment is slightly more polar than pigment **7** and shows a canthaxanthin-type electronic spectrum. These properties and the lability upon alkali treatment are consistent with structure **7** containing an additional keto group at C(4′).

The mass spectrum of the diacetate of **8** closely corresponds to that of **7** with all relevant peaks in the higher mass range shifted upward by 14 mass units.

The ^{1}H-NMR. spectrum of the diacetate of **8** again shows all signals expected for the 2,3-diacetoxy-4-keto-*β* end-group as given above and, in addition, the signals of the 4-keto-*β* end-group as in canthaxanthin (see exper. part).

The CD. spectrum of acetylated **8** is expected to be very similar to the one of acetylated **6**, since the additional *trans* substituent at C(2) is known to influence the CD. only very little [9] [10]. The measured spectrum shows, however, slight differences, which are probably caused by some decomposition (by light?) during the CD. measurement. The ^{1}H-NMR. which was repeated after the CD. measurement also indicated a partial decomposition of the sample. Despite these facts we conclude from the remaining resemblance to the CD. of **6** that the configuration at C(3) is the same. Together with the known relative configuration of the substituents at C(2) and C(3) this means that the most likely absolute configuration of **8** is (2*R*,3*S*). This structure also has not been reported before [7].

*(2*R,3S,2′R,3′R*)-2,3,2′,3′-Tetrahydroxy-β,β-caroten-4-one* (**9**). This compound is identical with the main pigment of the parent strain of *Rhizobium lupini* and was characterized by the criteria reported previously [1].

In the CD. spectrum of the tetraacetate of **9** the signs and positions of the maxima are identical to those of synthetic (3*S*,3′*R*)-adonixanthin ((3*S*,3′*R*)-3,3′-dihydroxy-*β*,*β*-caroten-4-one) [6]. Since the configuration at C(3) determines the signs of the CD. bands whereas an additional *trans*-substituent at C(2) in a related structure has no influence [9] [10] we conclude that the absolute configurations of pigment **9** at C(3) and C(3′) are identical to those in (3*S*,3′*R*)-adonixanthin. Since the hydroxyl groups at C(2) and C(3) as well as at C(2′) and C(3′) are *trans* to each other the absolute configuration of **9** is, therefore, deduced as (2*R*,3*S*,2′*R*,3′*R*).

(2R, 3S, 2'R, 3'S)-2, 3, 2', 3'-Tetrahydroxy-β, β-carotene-4, 4'-dione (**10**). This carotenoid was found to be the main pigment of the mutant strain 1-207. In the parent strain it occurred only in trace amounts. On adsorption chromatography it was slightly more polar than pigment **9**. This increased polarity and the canthaxanthin-type chromophore suggested an additional keto group at C(4').

The MS. of peracetylated **10** shows the expected fragmentation pattern. A minute molecular ion peak at *m/z* 796 is related to the fragments at 736 and 676 by elimination of one and two molecules of acetic acid, and to the fragments at 634 and 592 by elimination of two acetic acids plus one and two ketenes. In the middle of the mass spectrum fragments at *m/z* 245 and 203 are found as in the spectra of pigments **7** and **8**.

The ^{1}H-NMR. spectrum of tetraacetylated **10** reflects the high symmetry of the molecule since only one kind of end-group, the 2,3-*trans*-diacetoxy-4-keto-β end-group is present with all chemical shifts and coupling constants identical to those given above [1]. Since about 3 mg of this compound were available a ^{1}H-broad band decoupled ^{13}C-NMR. spectrum could be obtained which fully confirms the proposed structure. The chemical shifts and assignments are given in the experimental part.

The general shape of the CD. spectrum of the tetraacetate of **10** resembles that of (3*S*,3'*S*)-astaxanthin [3] and its diacetate. Differences at 280 and 380 nm are probably due to some decomposition during the measurement. Since the structure of **10** is unambiguously derived from NMR. and mass spectroscopic data and since its CD. spectrum is much more similar to that of (3*S*,3'*S*)-astaxanthin than that of its enantiomer, we conclude that **10** has (3*S*,3'*S*)-configuration. The configurations at C(2) and C(2') then follow again from the relative configurations of the substituents derived from the large coupling constants $J_{2,3}=J_{2',3'}=12$ Hz (axial position of the protons) as (2*R*,2'*R*). This carotenoid has not so far been found in nature [7].

The CD. spectrum of the previously identified pigment **11** (denoted as II in [1]) is virtually identical with that of (3*R*,3'*R*)-zeaxanthin [3]. Analogous reasons as given for compound **9** lead to the suggested absolute configurations of **11** as (2*R*,3*R*,2'*R*,3'*R*).

Discussion. – The carotenoid pattern of the mutant 1-207 is considerably more complex than that of the parent strain of *Rhizobium lupini* and the site of mutation in the carotenogenic pathway is not easily defined. The parent strain was shown to contain the 2,3,2',3'-tetrahydroxy-(**11**) and 2,3,2',3'-tetrahydroxy-4-keto structures (**9**) as the main carotenoids (about 45% each) [1]. The main pigment of the mutant strain is the 2,3,2',3'-tetrahydroxy-4,4'-dioxo derivative **10**. Furthermore, non-carbonylated components – apart from β,β-carotene – are almost absent in the mutant, less hydroxylated monooxo and dioxo derivatives, even echinenone and canthaxanthin, being present. From these observations we conclude that by an unknown mechanism carbonylation is favoured over hydroxylation.

The substitution pattern in the β end-groups of the carotenoids of this mutant and of another mutant defective in the carbonylation (to be published) furthermore suggests that (i) the hydroxylation at C(3) preceeds the hydroxylation at

C(2) in the biosynthetic sequence because 2-hydroxy or 2-hydroxy-4-keto end-groups have never been observed, and (ii) hydroxylation at C(3) and C(2) is prevented when a keto group is already present at C(4).

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Experimental Part

Culture. – *Rhizobium lupini* 1-250 and the mutant strain 1-207 were provided by Prof. *W. Heumann*. The cells were grown in 0.8% nutrient broth *(Merck)* in 500 ml Erlenmeyer flasks on a shaker at 30° under illumination with three fluorescent tubes (*Osram*-L 40W/25-1).

Isolation and chemical methods. – Extraction and isolation of carotenoids by column and thin layer chromatography and the chemical methods used for the characterization of the structures were essentially as described in [1].

Instrumentation. – The CD. spectra (nm) were recorded on a dichrographe Mark II *(Jobin-Yvon)* with a 450 watt Xe arc; see also [3]. Since the concentrations of the solutions were unknown, the $\Delta\varepsilon$ values are given relative to the strongest maximum in each spectrum. The 270 MHz ^{1}H-NMR. *Fourier* transform spectra in $CDCl_3$ (δ ppm, J Hz) were run on a HX-270 FT NMR. spectrometer (*Bruker*-Spectrospin). The ^{1}H-decoupled ^{13}C-NMR. spectrum of **10** (δ ppm) was measured on the same instrument at 68 MHz in 0.2 ml $CDCl_3$ (5 mm insert). The sample quantities available were 0.02–3 mg. For further details see [1]. The MS. were taken at 70 eV on a MS-9 from *AEI* (Manchester) using a direct introduction probe.

Spectroscopic data. – *β,β-Carotene* (**1**). Pigment yield about 0.8 mg. – VIS. (ethanol): 429, 449, 477. *β,β-Caroten-4-one* (**2**). Pigment yield about 0.8 mg. – VIS. (ethanol): 466 nm; reduced ($NaBH_4$): 429, 449, 477. – MS.: 550 (100, *M*); 458 (9).

β,β-Carotene-4,4′-dione (**3**). Pigment yield about 0.2 mg. – VIS. (ethanol): 477; reduced ($NaBH_4$) 429, 449, 477. – MS.: 564 (100, *M*); 472 (6).

(3S)-3-Hydroxy-β,β-caroten-4-one (**4**). Pigment yield about 0.3 mg. – VIS. (ethanol): 466; reduced ($NaBH_4$): 429, 449, 477. – CD. of acetylated **4** (CH_2Cl_2): 366 (−0.38), 306 (−1.00), 262 (0.63), 237 (−0.76). – ^{1}H-NMR. of acetylated **4**: 1.030 (*s*, 6 H, *gem* H_3C–C(1′)); 1.229 and 1.351 (2 *s*, 3 H each, *gem* H_3C–C(1); 1.723 (*s*, 3 H, H_3C–C(5′)); 1.907 (*s*, 3 H, H_3C–C(5)); ~1.983 and 1.998 (2 *s*, 'in-chain' H_3C); 2.193 (*s*, 3 H, H_3CCOO–C(3)); 5.526 (*d*×*d*, J = 13 and 6.5, 1 H, H(ax)–C(3)); 6.148 (*d*?, H–C(10′)); 6.138 (*A*-part of *AB*-type spectrum, H–C(8′)); 6.180 (*B*-part, H–C(7′)); 6.193 (*d*?, H–C(7)); 6.296 (*d*, J ~ 11.5, H–C(10)); 6.355 (*d*, J ~ 15, H–C(12′)); 6.410 (*d*, J ~ 16, H–C(8)); 6.448 (*d*, H ~ 15, H–C(12)); 6.58–6.70 (*m*, 4 H, H–C(11,11′), H–C(15,15′)). Judged by the integration curve H–C(14,14′) should absorb near 6.26 ppm as expected. – MS. of acetylated **4**: 608 (36, *M*); 566 (14); 548 (100).

(2R,3R)-β,β-Carotene-2,3-diol (**5**). Pigment yield about 0.1 mg. – VIS. (ethanol): 429, 449, 477. – ^{1}H-NMR. of acetylated **5**: 1.000 (*s*, 3 H, H_3C–C(1)); 1.029 (*s*, 6 H, *gem* H_3C–C(1′)); 1.081 (*s*, 3 H, H_3C–C(1)); 1.701 (*s*, 3 H, H_3C–C(5)); 1.720 (*s*, 3 H, H_3C–C(5′)); ~1.974 (~*s*, 12 H, 'in-chain' H_3C); 2.035 and 2.097 (*s*, 3 H each H_3CCOO–C(2) and C(3)); 5.040 (*d*, 1 H, H(ax)–C(2)); ~5.14 (*m*, 1 H, H(ax)–C(3)); 6.01 (*d*, J ~ 16, H–C(7)); ~6.11 (*d*?, H–C(8)); ~6.14 (H–C(7′) and H–C(8′)); 6.36 (*d*, J ~ 15, H–C(12′)); 6.38 (*d*, J ~ 15, H–C(12)); 6.57-6.73 (*m*, 4 H, H–C(11,11′) and H–C(15,15′)). – MS. of peracetylated **5**: 652 (100, *M*); 592 (10); 560 (12); 500 (5).

(3S)-3-Hydroxy-β,β-carotene-4,4′-dione (**6**). Pigment yield about 0.3 mg. – VIS. (ethanol): 477; reduced form of **6** ($NaBH_4$): 429, 449, 477. – CD. of acetylated **6** (CH_2Cl_2): 321 (−1.00), 279 (0.68), 247 (−0.99), 228 (0.41). – ^{1}H-NMR. of acetylated **6**: 1.196 (*s*, 6 H, *gem* H_3C–C(1′)); 1.230 and 1.352 (2 *s*, 3 H each, *gem* H_3C–C(1)); 1.85 (*t*, 2 H, H–C(2′)); 1.874 and 1.906 (*s*, 3 H each, H_3C–C(5′) and C(5)); *ca.* 2.00 (br., 'in-chain' H_3C); 2.193 (*s*, 3 H, H_3CCOO–C(3)); 2.51 (*t*, J ~ 6, ~2 H, H–C(3′)); ~5.53 (*d*×*d*, J ~ 13 and 6.5, H(ax)–C(3)); 6.21 (*d*?, H–C(7)); 6.24 (*d*?, H–C(7′)); 6.24–6.36 (*m*, H–C(10,10′) and H–C(14,14′)); 6.36 (*d*, J ~ 16, H–C(8′)); 6.41 (*d*?, H–C(8)); 6.43 (*d*, J ~ 15, H–C(12′)); 6.45 (*d*, J ~ 15, H–C(12)); 6.59-6.73 (*m*, H–C(11,11′) and H–C(15,15′)). The spectrum is identical with that of a synthetic (racemic) sample [5] [6]. – MS. of acetylated **6**: 622 (25, *M*); 580 (14); 562 (100).

(2R,3S)-2,3-Dihydroxy-β,β-caroten-4-one (**7**). Pigment yield about 0.3 mg. – VIS. (ethanol): 466 nm; reduced ($NaBH_4$): 429, 449, 477. – ^{1}H-NMR. of acetylated **7** (relevant signals only, sample slightly impure): 1.030 (*s*, *gem* $H_3C-C(1')$); 1.147 and 1.319 (2 *s*, *gem* $H_3C-C(1)$); 1.723 (*s*, $H_3C-C(5')$); 1.914 (*s*, $H_3C-C(5)$); *ca.* 1.984 and 2.003 (br. *s*, 'in-chain' H_3C); 2.124 and 2.169 (*s*, $H_3CCOO-C(2)$ and C(3)); 5.325 (*d*, $J\sim12$, H–C(2)); 5.533 (*d*, $J\sim12$, H–C(3)). In the olefinic range the following protons could be assigned: 6.15 ($\sim$*s*, H–C(7') and H–C(8')); 6.18 (*d?*, H–C(7)); 6.40 (*d*, $J\sim16$, H–C(8)); 6.46 (*d*, $J\sim15$, H–C(12)). – MS. of peracetylated **7**: 666 (1, *M*); 648 (3); 606 (18); 564 (33); 245 (22); 203 (100).

(2R,3S)-2,3-Dihydroxy-β,β-carotene-4,4'-dione (**8**). Pigment yield about 0.3 mg. – VIS (ethanol): 477 nm; reduced ($NaBH_4$): 429, 449, 477. – CD. of acetylated **8** (dioxane): 311 (−0.62), 280 (0.08), 244 (−1.00), 226 (−0.06 neg. min.). – ^{1}H-NMR. of diacetylated **8**: 1.146 and 1.319 (2 *s*, 3 H each, *gem* $H_3C-C(1)$); 1.195 (*s*, 6 H, *gem* $H_3C-C(1')$); 1.85 (*t?*, $J\sim6$, $H_2-C(2')$); 1.876 (*s*, 3 H, $H_3C-C(5')$); 1.913 (*s*, 3 H, $H_3C-C(5)$); $\sim$1.994 and 2.006 (2 *s*, 12 H, 'in-chain' H_3C); 2.125 and 2.169 (2 *s*, 3 H each, $H_3CCOO-C(2)$ and C(3)); 2.51 (*t*, $J\sim6$, $H_2C(3')$); 5.326 (*d*, $J=12$, 1 H, H(ax)–C(2)); 5.534 (*d*, $J=12$, 1 H, H(ax)–C(3)); 6.18 (*d*, $J\sim16$, H–C(7)); 6.23 (*d*, $J=16$, H–C(7')); $\sim$6.28 (*d?*, H–C(10')); 6.31 (*d?*, H–C(10)); 6.37 (*d*, $J\sim16$, H–C(8')); 6.39 (*d*, $J\sim16$, H–C(8)); 6.43 (*d*, $J\sim15$, H–C(12')); 6.46 (*d*, $J\sim15$, H–C(12)); 6.65 and 6.66 (*d*×*d?*, H–C(11,11')); *ca.* 6.67 (*m*, H–C(15,15')). – MS. of peracetylated **8**: 680 (1, *M*); 662 (2); 620 (35); 578 (85); 245 (22); 203 (100).

(2R,3S,2'R,3'R)-2,3,2',3'-Tetrahydroxy-β,β-caroten-4-one (**9**). Pigment yield about 0.3 mg. – For the ^{1}H-NMR. and mass spectroscopic data of this compound see [1]. – CD. of acetylated **9** (dioxane): 350 (0.20), 300 (−1.00), 260 (0.40), 236 (−0.65).

(2R,3S,2'R,3'S)-2,3,2',3'-tetrahydroxy-β,β-carotene-4,4'-dione (**10**). Pigment yield about 5 mg. – VIS. (ethanol): 477 nm; reduced ($NaBH_4$): 429, 449, 477. – CD. of acetylated **10** (CH_2Cl_2): 373 (−0.25), 317 (−0.70), 282 (−0.06 neg. min.), 248 (−1.00). – ^{1}H-NMR. of peracetylated **10**: 1.146 and 1.320 (2 *s*, 6 H each, *gem* $H_3C-C(1)$ and $-C(1')$); 1.912 (*s*, 6 H, $H_3C-C(5,5')$); 1.995 and 2.007 (2*s*, 12 H together, 'in-chain' H_3C); 2.125 and 2.169 (2*s*, 6 H each, $H_3CCOO-C(2,2')$ and C(3,3')); 5.326 (*d*, $J=12$, 2 H, H(ax)–C(2,2')); 5.536 (*d*, $J=12$, H(ax)–C(3,3')); 6.190 (*d*, $J=16.3$, 2 H, H–C(7,7')); *ca.* 6.31 (*m*, H–C(10,10') and H–C(14,14')); 6.393 (*d*, $J\sim16$, H–C(8,8')); 6.460 (*d*, $J=15$, H–C(12,12')); 6.656 (*d*×*d*, $J=14.7$ and 11.5, H–C(11,11')); *ca.* 6.68 (*m*, H–C(15,15')). – ^{13}C-NMR. of peracetylated **10** (the assignments are based on the data for related compounds [11]): 12.60 and 12.83 ($CH_3-C(9,9')$ and C(13,13')); 14.03 ($CH_3-C(5,5')$); 20.61 and 20.72 (acetyl); 21.84 and 25.50 ($CH_3-C(1,1')$); 41.85 (C(1,1')); 73.63 and 74.42 (C(2,2') and C(3,3')); 122.34 (C(7,7')); 124.60 (C(11,11')); 129.34 (C(5,5')); 130.84 (C(15,15')); 133.99 and 134.29 (C(14,14') and C(9,9')); 135.48 (C(10,10')); 136.76 (C(13,13')); 140.07 (C(12,12')); 142.66 (C(8,8')); 159.69 (C(6,6')); 169.88 and 170.30 (2×COO); 191.25 (C(4,4')). – MS. of peracetylated **10**: 796 (0.5, *M*); 736 (6); 676 (60); 634 (100); 592 (50); 245 (20); 203 (80).

(2R,3R,2'R,3'R)-2,3,2',3'- tetrahydroxy-β,β-carotene (**11**). ^{1}H-NMR. and mass spectroscopic data of the tetraacetate are given in [1]. – CD. of the tetraacetate of **11** (ether/isopentane/ethanol 5:5:2): 343 (0.06), 280 (−1.00), 243 (0.52), 220 (−0.51), 210 (0.37).

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