63. Synthesis of Carotenoids in the Gliding Bacteria *Taxeobacter*: (all-*E*,2′*R*)-3-Deoxy-2′-hydroxyflexixanthin

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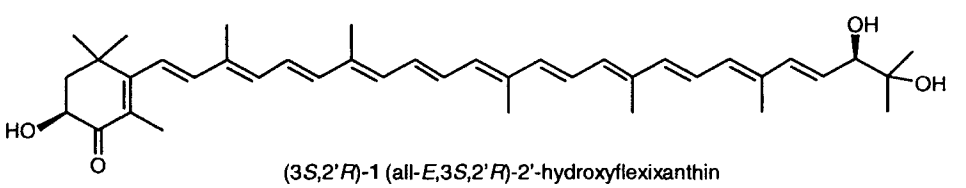
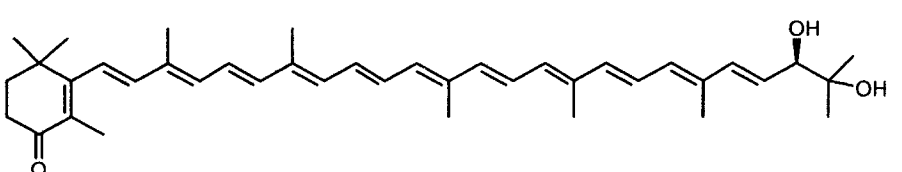
The C_{40}-carotenoid (all-*E*,2′*R*)-deoxy-2′-hydroxyflexixanthin (= 1′,2′-dihydroxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-4-one; (2′*R*)-**2**) was synthesized according to a $C_{15} + C_{10} + C_5 + C_{10} = C_{40}$ strategy. The chiral centre was introduced into the C_{10}-end group by the enantioselective *Sharpless* dihydroxylation. The four building blocks were coupled by applying four consecutive *Wittig* reactions. By comparison of the CD spectra of the synthetic (2′*R*)-**2** with those of **2** isolated from the gliding bacteria *Taxeobacter*, the configuration of natural **2** was determined as (2′*R*).

1. Introduction. – Carotenoids are not only encountered in many higher plants and in animals, they are also common in bacteria and archaea. The gliding bacteria *Taxeobacter* was found for the first time by *Reichenbach* [1] [2] in cultures of *E. coli*. Later, several different species of *Taxeobacter* have been isolated from soils [1] [2]. Recently, *Böhlendorf* and *Reichenbach* [3] identified 2′-hydroxyflexixanthin (= 3,1′,2′-trihydroxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-4-one; **1**) as major carotenoid of the dark-red-coloured bacteria and, in addition, also the previously unknown 3-deoxy-2′-hydroxyflexixanthin (= 1′,2′-dihydroxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-4-one; **2**) was isolated as minor pigment. However, the configuration at the asymmetric C-atoms of **1** and **2** was not established. The 2′-hydroxyflexixanthin (**1**) had been previously isolated by *Andrewes*



(3*S*,2′*R*)-**1** (all-*E*,3*S*,2′*R*)-2′-hydroxyflexixanthin

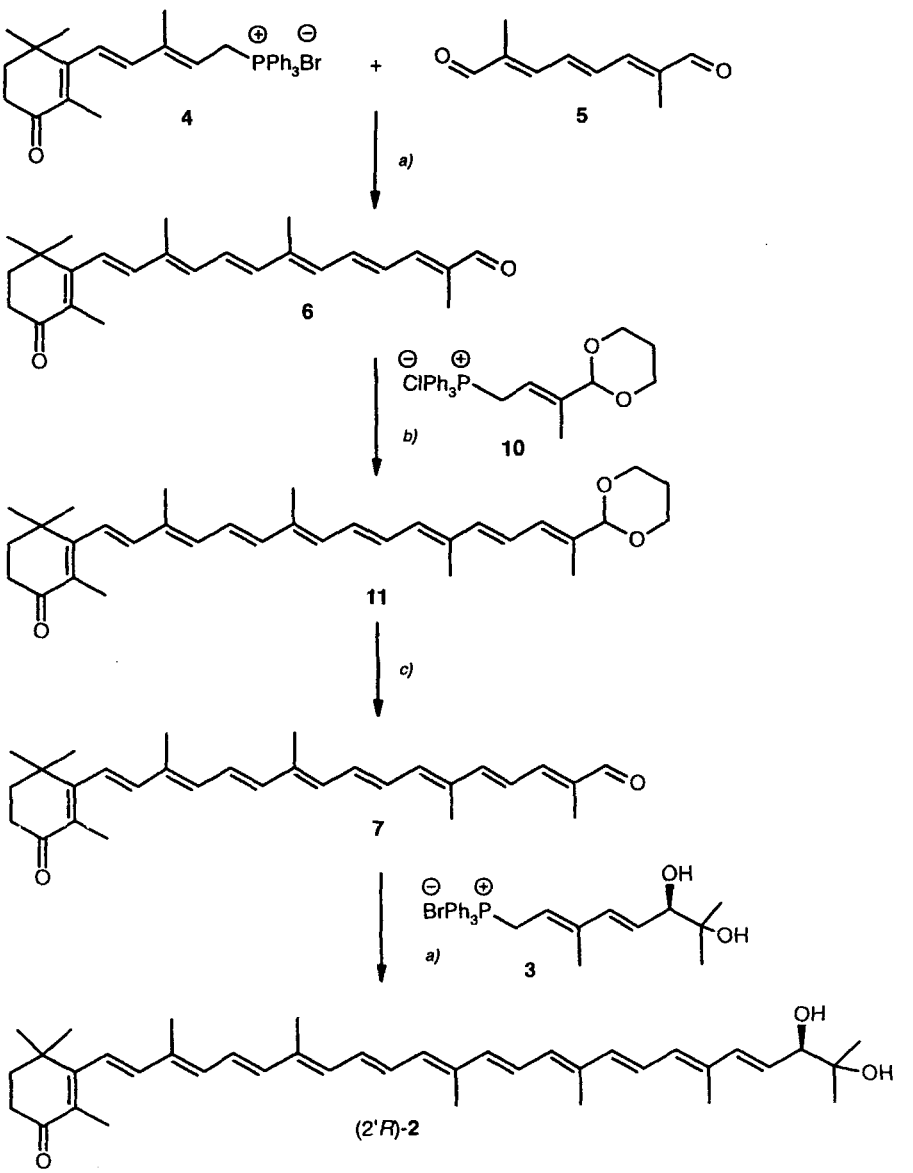


(2′*R*)-**2** (all-*E*, 2′*R*)-3-deoxy-2′-hydroxyflexixanthin

[1]) Diploma work and part of the Ph.D. thesis of *C.B.*

et al. from *Flexibacter* (strain NIVA BRG-64), and, based on comparison of the CD spectra with the spectra of plectaniaxanthin (= 3′,4′-didehydro-1′,2′-dihydro-β,ψ-carotene-1′,2′-diol), the (3*S*,2′*S*)-configuration was postulated [4]. In view of the structure elucidation, especially by comparison of the CD spectra of the natural and the synthetic compounds, we report in the following the synthesis of (all-*E*,2′*R*)-3-deoxy-2′-hydroxyflexixanthin ((2′*R*)-**2**).

Scheme 1



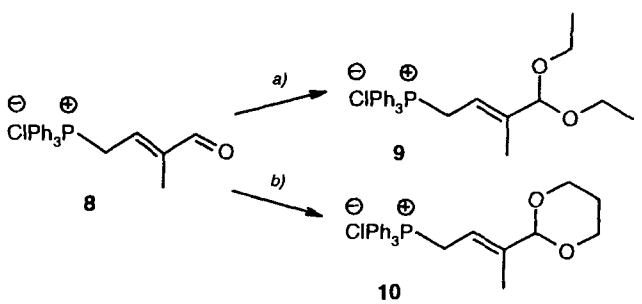
a) 1,2-Ethoxybutane *b*) NaOMe, MeOH. *c*) 5% H_2SO_4, Et_2O/H_2O.

2. Results and Discussion. – For the synthesis of optically active **2**, the strategy $C_{15} + C_{10} + C_5 + C_{10} = C_{40}$ was selected (*Scheme 1*). The C_{10}-ψ-end group **3** containing the chiral centre at the C(2) position was synthesized in eight steps using the pathway of *Traber* and *Pfander* [5].

The C_{15}-phosphonium salt **4**, the end group of the commercially available carotenoid canthaxanthin (= β,β-carotene-4,4'-dione), was kindly provided by *F. Hoffmann-La Roche Ltd.*, Basel. The synthesis of **4** is described by *Kienzle* and *Meyer* [6] and *Widmer et al.* [7].

First, **4** was elongated by a *Wittig* reaction in epoxybutane with the C_{10}-dial **5** (*Scheme 1*) to give the C_{25}-apocarotenal **6** accompanied by a small amount (5%) of canthaxanthin, resulting from the double *Wittig* reaction of **5**. By column chromatography (silica gel), **6** was obtained in 51% yield. For the elongation of **6** to the C_{30}-buidling block **7**, the C_5-phosphonium salt **8** was protected using a method described by *Freyschlag et al.* [8] [9] to give the diethyl acetal **9** (*Scheme 2*). However, the coupling of **6** with **9** gave a complex mixture of products, partially due to the cleavage of the protection group. Therefore, the cyclic acetal **10** was prepared which was used without purification for the reaction with the C_{25}-synthon **6**. The resulting C_{30}-acetal **11** was transformed to the building block **7** in an overall yield of 42% (referred to **6**) by careful treatment with 5% H_2SO_4 for 30 min in a vigorously stirred H_2O/Et_2O mixture. For the synthesis of (2'*R*)-**2**, the C_{30}-synthon **7** and the phosphonium salt **3** were reacted in 1,2-epoxybutane to give the C_{40}-carotenoid in a yield of *ca.* 13% after crystallization from benzene/hexane.

Scheme 2



a) $CH(OEt)_3$, MeOH, TsOH. *b*) Propane-1,3-diol, MeOH, TsOH.

3. Spectroscopic Studies. – The UV/VIS spectra of (2'*R*)-**2** exhibits a λ_{max} at 474 nm (in hexane) with almost no fine structure due to the carbonyl function at C(4). This value corresponds to the value given for deoxyflexixanthin (= 1'-hydroxy-3',4'-didehydro-1',2'-dihydro-β,ψ-caroten-4-one) by *Coman* and *Weedon* [10]. The mass spectrum shows the molecular ion at *m/z* 582, which is in agreement with the constitution of **2**. NMR investigations of (2'*R*)-**2**, comprising $^1H,^{13}C$, DEPT, $^1H,^1H$-COSY and H,C correlations experiments, allow the assignment of all signals unambiguously (see *Exper. Part*).

The CD spectrum of (2'*R*)-**2** in Et_2O/isopentane/EtOH 5:5:2 (EPA 5:5:2) exhibited a strong temperature dependence. At $-100°$, the spectrum shows a main extremum at 285 ($\Delta\varepsilon = -33$; *Fig.*). The spectrum is almost identical to the spectrum obtained from

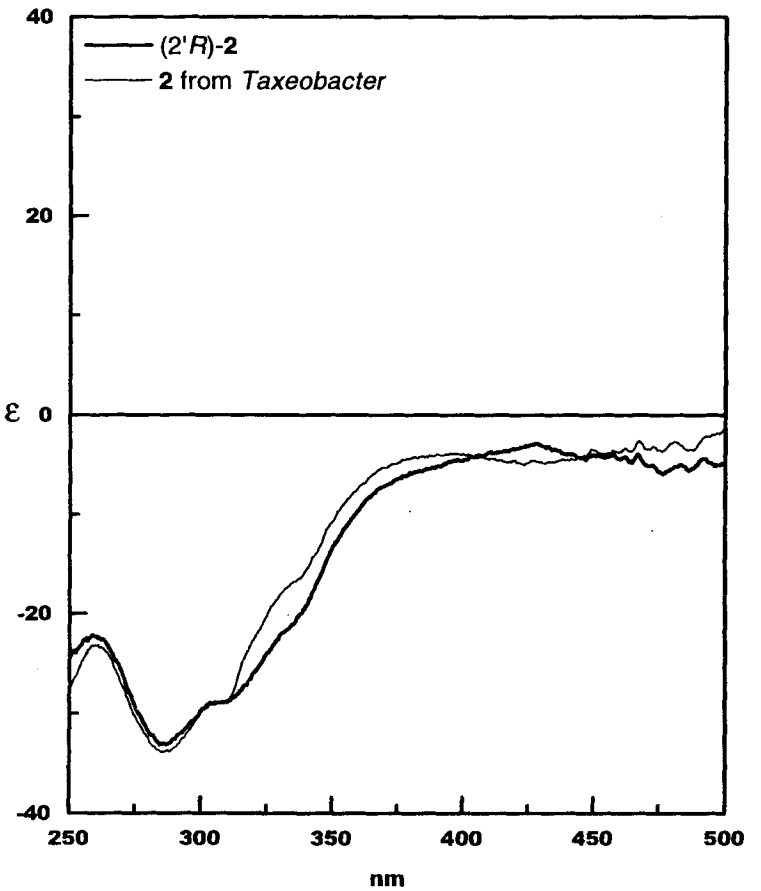


Figure. *Comparison of CD spectra* (EPA 5:5:2, −100°) *of synthetic (2'*R*)-***2** *and natural* **2**

natural **2**. Therefore, the structure of the carotenoid isolated from *Taxeobacter* was established as (all-*E*,2'*R*)-3-deoxy-2'-hydroxyflexixanthin ((2'*R*)-**2**).

The synthesis of the main carotenoid of *Taxeobacter*, (all-*E*,3*S*,2'*R*)-2'-hydroxyflexixanthin ((3*S*,2'*R*)-**1**), by a $C_{15} + C_{10} + C_{15} = C_{40}$ strategy is in progress.

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Experimental Part

1. *General.* All experiments were carried out under N_2 or Ar. Solvents were distilled prior to use or purchased in HPLC quality. TLC: silica gel $25F_{254}$ (*Macherey-Nagel*). Column chromatography: silica gel *60* (*Merck*, 0.040–0.063 mm). M.p.: *Büchi 510*; not corrected. $[\alpha]_D$: *Perkin-Elmer-241* polarimeter. UV/VIS Spectra: *Perkin-Elmer-554* spectrometer; λ_{max} in nm. CD Spectra: *Jasco 500*, wavelength in nm ($\Delta\varepsilon$). IR Spectra: *Perkin-Elmer-782* spectrometer; ν in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker AC-300* (300 and 75.5 MHz resp.), *Bruker AM-400*

(400 and 100.6 MHz, resp.), in $CDCl_3$; chemical shifts in ppm as δ values relative to $CDCl_3$ (= 7.26 ppm), J in Hz. Mass spectra: *Varian MAT CH-44s*; *m/z* (rel. intensity in %); ionization energy 70 eV.

2. *(6R,4E)-(6,7-Dihydroxy-3,7-dimethyloct-2,4-dien-1-yl)triphenylphosphonium Bromide* (**3**) was synthesized according to [5].

3. *4-Oxo-12'-apo-β-caroten-12'-al* (**6**). To a soln. of **5** (340 mg, 2.1 mmol) in 1,2-epoxybutane (= 2-ethyloxirane, 20 ml) was added **4** (1.39 g, 2.5 mmol) and the mixture heated to 60°. After 16 h, the deep-red soln. was evaporated at 40°. The crude product was dissolved in EtOH/H_2O 8:2 (10 ml) and stirred for 3 h at 60°. After cooling slowly to r.t., the product was crystallized in the deep-freezer at −20° overnight. The crystals were separated by filtration and washed carefully with ice-cold MeOH. An additional crystallization was performed with the mother liquor. The combined crystals were dried under h.v. to give 460 mg (51%) of **6**. Deep-red crystals. M.p. 166°. UV/VIS (hexane): 273, 318, 422. IR (KBr): 1655*s* (C=O), 1610*m*, 1590*m*, 1570*m*, 1350*w*, 1330*w*, 1285*w*, 1185*m*, 970*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.18 (*s*, Me(16), Me(17)); 1.86 (*s*, Me(18)); 1.89 (*s*, Me(20')); 2.03 (*s*, Me(19)); 2.07 (*s*, Me(20)); 2.52 (*t*, J = 7, CH_2(3)); 6.2–6.5 (*m*, H–C(7), H–C(8), H–C(10), H–C(12), H–C(14)); 6.60–6.83 (*m*, H–C(11), H–C(15')); 6.98 (*d*, J = 10, H–C(14')); 7.05 (*dd*, J = 15, 10, H–C(15)); 9.42 (*s*, H–C(12')). MS: 364(21, M^+), 349(7), 332(11), 295(10), 277(38), 253(9), 215(14), 209(10), 203(22), 197(18), 183(23), 175(13), 171(26), 164(33), 157(72), 147(28), 135(41), 119(28), 105(28), 95(42), 91(44), 77(26), 69(21), 59(44), 55(20), 43(33), 28(35), 18(100).

4. *4-Oxo-8'-apo-β-caroten-8'-al* (**7**). To a soln. of **8** (534 mg, 1.44 mmol) in MeOH (30 ml), propan-1,3-diol (560 mg, 6 mmol) was added through a syringe, together with a few crystals of TsOH dissolved in MeOH. The mixture was stirred for 15 h at. r.t. After evaporation, the remaining thick oil was dissolved in CH_2Cl_2, and **10** was precipitated by adding THF. The white residue was separated by filtration and dissolved in MeOH (20 ml) without further purification. Then, **6** (362 mg) was added, and then NaOMe (60 mg) in MeOH (2 ml) was injected. After stirring at 40° for 18 h, the mixture was allowed to cool to r.t. H_2O (50 ml) was added and the mixture extracted with Et_2O (3 ×). The combined org. phases were washed twice with sat. NaCl soln., then dried (Na_2SO_4), and evaporated. The crude product was dissolved in Et_2O (10 ml), mixed with 0.5N H_2SO_4 (10 ml), and vigorously stirred at r.t. for 30 min. The H_2O phase was separated and extracted with Et_2O (3 ×). The combined org. phases were washed with sat. $NaHCO_3$ soln. (3 ×) and sat. NaCl soln. (2 ×), dried (Na_2SO_4), and evaporated. The product was crystallized from EtOH/H_2O 4:1 and dried under h.v. 260 mg (42%), referred to **6** of **7**. Deep-red crystals. M.p. 178°. UV/VIS (hexane): 289, 348, 451, 476. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.20 (*s*, Me(16), Me(17)); 1.89, 1.90 (2*s*, Me(18), Me(19')); 1.92 (*s*, Me(19)); 1.99–2.01 (*s*, Me(20), Me(20')); 2.52 (*t*, J = 7, CH_2(3)); 6.1–6.5 (*m*, H–C(7), H–C(8), H–C(10), H–C(12), H–C(12'), H–C(14), H–C(14')); 6.55–6.80 (*m*, H–C(11), H–C(11'), H–C(15), H–C(15')); 6.95 (*d*, J = 11, H–C(10')); 9.45 (*s*, H–C(8')). MS: 430 (10, M^+), 361(4), 296(5), 277(6), 215(13), 203(15), 197(13), 183(11), 171(11), 157(14), 147(15), 133(19), 119(16), 105(12), 95(11), 91(11), 69(10).

5. *(all-E,2'R)-3-Deoxy-2'-hydroxyflexixanthin* ((2'*R*)-**2**). To a soln. of **3** (408 mg, 0.8 mmol) in 1,2-epoxybutane (20 ml), **7** (200 mg, 0.4 mmol) was added. After stirring for 15 h at 60°, more **3** (200 mg) was added to the mixture, and after 8 and 14 h, even more (150 and 100 mg, resp.). The mixture was stirred for another 18 h, then cooled down to r.t., evaporated at 40°. The dark-red product was separated by column chromatography (silica gel 100 g), hexane/AcOEt 1:1 to 1:2 + 0.5% Et_3N. Crystallization from benzene/hexane yielded 36 mg (13%) of (2'*R*)-**2**. Deep-red needles. M.p. 204° (dec.). UV/VIS (hexane): 314, 474, 500. CD (EPA 5:5:2, −100°): 285(−33), 307(−29), 427(−3). ^{1}H-NMR (400 MHz, $CDCl_3$): 1.18 (*s*, Me(16')); 1.19 (*s*, Me(16), Me(17)); 1.24 (*s*, Me(17')); 1.83 (*t*, J = 6.56, CH_2(2)); 1.87 (*s*, Me(18)); 1.93 (*s*, Me(18')); 1.98 (*s*, Me(19'), Me(20), Me(20')); 2.00 (*s*, Me(19)); 2.12 (*d*, J = 2.43, OH); 2.50 (*t*, J = 6.56, CH_2(3)); 4.00 (*dd*, J = 7.51, 2.43, H–C(2')); 5.71 (*dd*, J = 15.52, 7.61, H–C(3')); 6.20 (*d*, J = 11.3, H–C(6')); 6.23 (*d*, J = 15.41, H–C(7)); 6.26 (*d*, J = 11.5, H–C(10')); 6.28 (*d*, J = 11.5, H–C(10)); 6.28 (*m*, H–C(14')); 6.37 (*d*, J = 15.41, H–C(8)); 6.38 (*d*, J = 15.52, H–C(4')); 6.39 (*d*, J = 14.61, H–C(8')); 6.40 (*d*, J = 15.0, H–C(12')); 6.43 (*d*, J = 15.00, H–C(12)); 6.59 (*dd*, J = 13.3, 14.61, H–C(7')); 6.65 (*dd*, J = 11.5, 15.00, H–C(11)); 6.65 (*m*, H–C(15)); 6.65 (*dd*, J = 11.5, 15.0, H–C(11')); 6.66 (*m*, H–C(15')). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.6(Me(19)); 12.8(Me(20), Me(20')); 12.9(Me(19')); 13.0(Me(18')); 13.8(Me(18)); 24.0(Me(16')); 26.5(Me(17')); 27.7(Me(16), Me(17)); 34.3(CH_2(3)); 36.7(C(1)); 37.4(CH_2(2)); 73.0(C(1')); 80.1(CH(2')); 124.0(CH(7)); 124.3(CH(7')); 124.5(CH(11)); 125.2(CH(11')); 126.3(CH(3')); 129.9(C(5)); 130.2(CH(15)); 130.7(CH(15')); 132.9(CH(14')); 133.1(CH(6')); 133.3(CH(10')); 133.7(CH(14), C(9')); 134.1(C(5')); 134.5(CH(10)); 134.7(C(9)); 136.1(C(13)); 136.4(C(13')); 138.1(CH(4')); 138.3(CH(12')); 138.7(CH(8')); 139.3(CH(12)); 141.3(CH(8)); 161.2(C(6)); 199.3(C(4)). MS: 582(19, M^+), 564(3), 548(2), 524(9), 476(32), 419(16), 326(14), 295(6), 283(7), 269(8), 263(6), 255(8), 247(7), 237(9), 223(16), 215(20), 209(26), 203(70), 197(27), 185(24), 169(23), 161(19), 145(51), 139(43), 133(82), 119(57), 105(69), 91(100), 81(30), 69(38), 59(54).

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