Bacterial Carotenoids. 50. * On the Structures of (3S)-Flexixanthin and (3S,2'S)-2'-Hydroxyflexixanthin

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Flexixanthin from Flexibacter sp. has been assigned structure I, Scheme 1, on the basis of chemical and spectroscopical characterization.1 The achiral derivative dehydroflexixanthin (2) then also characterized1 was later synthesized.2 Flexixanthin (I) was reisolated and 1H NMR analysis including spin decoupling confirmed the previous assignment. The CD spectrum of the chiral diacetate 3b, prepared by reducing flexixanthin (I) to the tetrol 3 followed by standard

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acetylation, was compared with that3 of (3R)-rubixanthin (4), Fig. 1; 3b and 4 possess monocyclic dodecaene and undecaene chromophores respectively. The similar Cotton effects of 3b and 4 permit the assignment of 3S-chirality for flexixanthin (Ia); formally4 (3S)-3,3'-dihydroxy-3', 4'-didehydro-1',2'-dihydro-β,γ-caroten-4-one).
The ca. 10 nm bathochromic displacement of the negative peak in the CD spectra of 3b compared to 4 is consistent with a similar shift in their visible spectra (λmax in hexane 461 nm for 4 and 473 nm for 3b), cf. Ref. 3.

Whereas marine animals frequently contain carotenoids with partly racemized α-ketol end groups E,5-7 organisms producing carotenoids de novo biosynthesize optically pure carotenoid α-ketols.8,9 The high Δε of flexixanthin (Ia, Fig. 1, Δε=-9.1) in comparison with that of (3S,3'S)-astaxanthin with two end groups E (Δε=14.4)10 and of the diacetate 3b (Δε=-9.1) in comparison with that of rubixanthin (4; Δε=-9.1)11 suggests a high enantiomeric purity of flexixanthin (Ia).

2'-Hydroxyflexixanthin from strain NIVA BRG-64 was assigned structure 5 from chemical and spectroscopic evidence,12 not including 1H NMR. 2'-Hydroxyflexixanthin was reisolated and 1H NMR analysis supported the previous assignment. The CD spectrum, Fig. 1, of the 2'-hydroxyflexixanthin derivative 6b may, according to the additivity hypothesis13 for carotenoids with identical chromophores, be considered as a composite of the contributions from each chiral end group.14 It is known that the Cotton effect of

![Fig. 1. CD spectra in EPA solution of ——— flexixanthin (Ia), ··· NaBH₄-reduced flexixanthin diacetate (3b), —— rubixanthin (4) and × NaBH₄-reduced 2'-hydroxyflexixanthin triacetate (6b).](image-url)
the β-end is determined by the chirality of 6b at C-3.\textsuperscript{15} The CD spectrum of 6b may be constructed by addition of the Cotton effect of the triacetate 3b and the mirror image of the Cotton effect of (2'R)-pectanixanthin-2'-ester (7)\textsuperscript{16} of known configuration.\textsuperscript{14} This permits the configurational assignment of 2'-hydroxyflexitanthxin (5a; (3S,2'S,3,1',2',trihydroxy-3',4'-didehydro-1',2'-dihydro-β,γ-caroten-4-one).

The enantiomeric purity of 2'-hydroxyflexitanthxin (5a) was examined by the camphaneate method.\textsuperscript{17,18} Chromatography of the camphaneate(s) in two different HPLC systems under conditions where the three diastereomeric camphaneates of (3S,3'S), (3R,3'R) and meso astaxanthin were well separated, suggested that the optical purity of 2'-hydroxyflexitanthxin (5a) was at least 98%. It is reasonable to assume the same optical purity also for flexitanthxin (1a).

Experimental. Biological material. Extracts of Flexibacter strain NIVA were available from an earlier study.\textsuperscript{12} Materials and methods. Standard procedures were used.\textsuperscript{19} Reactions were carried out on the μg scale (<1 mg). NaBH₄-reductions and acetylations gave >80% yields. Visible spectra were recorded in Me₂CO; CD spectra in EPA (ethanol-isopentane-ethanol 5:5:2) on a Roussel-Jouan Dichrographe.

Flexitanthxin (1a) was isolated and purified by TLC on Kieselgel 60 F₂₅₄ developed with Me₂CO-hexane (30:70), Rₜ = 0.58, followed by rechromatography with EtOAc-hexane (45:55), Vis. λₚₚ max nm (455), 460, (505). \textsuperscript{1}H NMR (CDCl₃) δ 1.99 (five methyls, four in-chain and one end-of-chain in β-ring); 1.95 (one-in-chain/end-of-chain methyl), 1.32, 1.21 (non-equivalent gem. methyls on β-end); 1.24 (two methyls attached to tert. hydroxyl); 2.32 (d, J = 7 Hz, C-2' methylene; confirmed by irradiation of H-3' at δ 5.80); 4.31 (dd, J₈,₉,₁₀ = 14 Hz, J₉,₁₀,₁₁ = 6 Hz, H-3); 5.80 (m, J₃,₂ = 7 Hz, J₆,₄ = 16 Hz, H-3' end-of-chain olefinic proton). MS m/z 582 (M⁺, 52%), M = 18 (9%), M = 587 (7%), M = 92 (5%), M = 106 (100%).

Reduced flexitanthxin (3). 1a in MeOH was treated with NaBH₄. TLC on Kieselgel developed with Me₂CO-hexane (40:60) gave 3 in a virtually quantitative yield, Vis. λₚₚ max nm 449, 473, 504.

Reduced flexitanthxin diacetate (3b). 3 Was acetylated and the reaction mixture was worked up in the usual manner. TLC on Kieselgel with Me₂CO-hexane (40:60) gave two products with identical Vis. and MS; Rₜ = 0.38 (minor), 0.43 (major); presumably cis and trans C-3,4 diesters. Vis λₚₚ max nm 448, 473, 504. MS m/z 668 (M⁺, 59%), M = 18 (5%), M = 58 (4%), M = 60 (8%), M = 92 (6%), M = 106 (100%), M = 118 (17%), M = 120 (11%); CD Fig. 1, recorded for the major 3,4-trans (?) isomer, presumed to be the same as for the 3,4-cis isomer.\textsuperscript{13}

2'-Hydroxyflexitanthxin (5a). Rₜ = 0.48 Kieselgel Me₂CO-hexane (40:60). Vis. λₚₚ max nm (454), 480, 505. \textsuperscript{1}H NMR (CDCl₃) δ 1.99 (five methyls, four in-chain and one end-of-chain in β-ring); 1.95 (one in-chain/end-of-chain methyl), 1.32, 1.21 (non-equivalent gem. methyls on β-end); 4.31 (dd, assignments same as for 1a); 4.00 (d, J = 7 Hz, H-2'); 5.71 (dd J₅,₂ = 7 Hz, J₅,₆ = 16 Hz, H-3'). MS m/z 598 (M⁺, 77%), M = 16 (15%), M = 18 (26%), M = 34 (23%), M = 36 (15%), M = 58 (9%), M = 60 (13%), M = 76 (8%), M = 90 (8%), M = 92 (5%), M = 106 (100%), M = 122 (13%), M = 124 (20%).

Reduced 2'-hydroxyflexitanthxin (6). 6 Was obtained by NaBH₄ reduction of 5a. Vis. λₚₚ max nm

Reduced 2'-hydroxyflexanthin triacetate (6b). Acetylation of 6 provided 6b. Vis. $\lambda_{\text{max}}$ nm, 448, 473, 504; MS m/z 726 (M$^+$, 15 %), M−60 (100 %), M−76 (21 %), M−106 (21 %), M−120 (15 %), M−156 (61 %), M−172 (36 %).

2’Hydroxyflexanthin (5a) dicamphaneate. The camphane was prepared from (−)-camphanol chloride by the general procedure. HPLC (Perkin-Elmer Series 2LC equipped with LC-85 spectrophotometric detector at 491 nm) on Spheri-5 silica, 5 $\mu$m (25×0.46 cm) column; eluted with EtOAc-hexane (35+65), flow 2 ml/min, showed one peak $R_f$=7.68’ with a small inflexion at $R_f$=6.88’ under conditions where the diastereomeric astaxanthin camphanates were well separated. On Cyano Spheri-5 column (25×0.46 cm) eluted with hexane-isopropyl acetate−Me$_2$CO (72+16+12), flow 2 ml/min, $R_f$=8.83’ (2 %) and $R_f$=10.15’ (98 %) under conditions where the all-trans diastereomeric astaxanthin camphanates had $R_f$ 6.96’, 8.32’ and 10.15’ with baseline separation.

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