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

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Flexixanthin from *Flexibacter* sp. has been assigned structure *1*, Scheme 1, on the basis of chemical and spectroscopical characterization.[1] The achiral derivative dehydroflexixanthin (*2*) then also characterized[1] was later synthesized.[2] Flexixanthin (*1*) was reisolated and ^{1}H NMR analysis including spin decoupling confirmed the previous assignment. The CD spectrum of the chiral diacetate *3b*, prepared by reducing flexixanthin (*1*) to the tetrol *3* followed by standard acetylation, was compared with that[3] of (3*R*)-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 3*S*-chirality for flexixanthin (*1a*; formally[4] (3*S*)-3,1′-dihydroxy-3′, 4′-didehydro-1′,2′-dihydro-β,ψ-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 (λ_{max} 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 $\Delta\varepsilon$ of flexixanthin (*1a*, Fig. 1, $\Delta\varepsilon=-9.1$) in comparison with that of (3*S*,3′*S*)-astaxanthin with two end groups E ($\Delta\varepsilon=14.4$)[10] and of the diacetate *3b* ($\Delta\varepsilon=-9.1$) in comparison with that of rubixanthin (*4*; $\Delta\varepsilon=-9.1$)[11] suggests a high enantiomeric purity of flexixanthin (*1a*).

2′-Hydroxyflexixanthin from strain NIVA BRG-64 was assigned structure *5* from chemical and spectroscopic evidence,[12] not including ^{1}H NMR. 2′-Hydroxyflexixanthin was reisolated and ^{1}H NMR analysis supported the previous assignment. The CD spectrum, Fig. 1, of the 2′-hydroxyflexixanthin derivative *6b* may, according to the additivity hypothesis[13] 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

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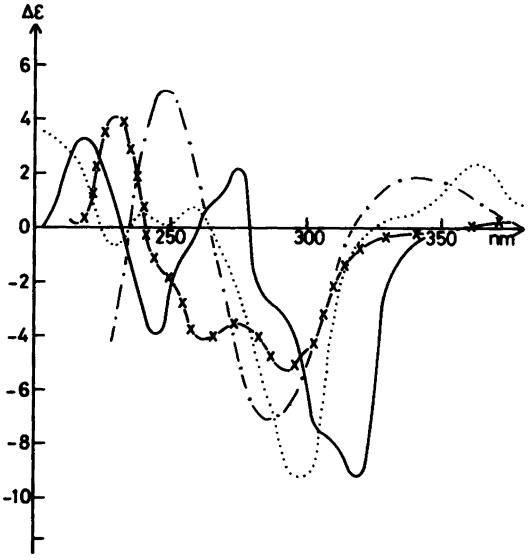

Fig. 1. CD spectra in EPA solution of ——— flexixanthin (*1a*), · · · $NaBH_4$-reduced flexixanthin diacetate (*3b*), —·— rubixanthin (*4*) and × $NaBH_4$-reduced 2′-hydroxyflexixanthin triacetate (*6b*).

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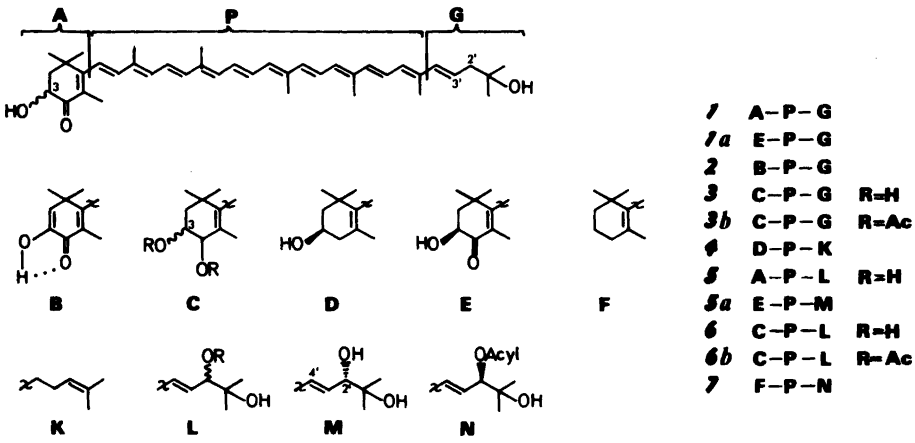

1 A-P-G
1a E-P-G
2 B-P-G
3 C-P-G R=H
3b C-P-G R=Ac
4 D-P-K
5 A-P-L R=H
5a E-P-M
6 C-P-L R=H
6b C-P-L R=Ac
7 F-P-N

the β-end is determined by the chirality of *6b* at C-3.[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*)-plectaniaxanthin-2′-ester (*7*)[16] of known configuration.[14] This permits the configurational assignment of 2′-hydroxyflexixanthin (*5a*; (3*S*,2′*S*)-3,1′,2′-trihydroxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-4-one).

The enantiomeric purity of 2′-hydroxyflexixanthin (*5a*) was examined by the camphanate method.[17,18] Chromatography of the camphanate(s) in two different HPLC systems under conditions where the three diastereomeric camphanates of (3*S*,3′*S*)-, (3*R*,3′*R*)- and meso astaxanthin were well separated, suggested that the optical purity of 2′-hydroxyflexixanthin (*5a*) was at least 98 %. It is reasonable to assume the same optical purity also for flexixanthin (*1a*).

Experimental. Biological material. Extracts of *Flexibacter* strain NIVA were available from an earlier study.[12]

Materials and methods. Standard procedures were used.[19] Reactions were carried out on the μg scale (<1 mg). $NaBH_4$-reductions and acetylations gave >80 % yields. Visible spectra were recorded in Me_2CO; CD spectra in EPA (ether-*iso*pentane-ethanol 5:5:2) on a Roussel-Jouan Dichrographe.

Flexixanthin (*1a*) was isolated and purified by TLC on Kieselgel 60 F_{254} developed with Me_2CO-hexane (30+70), R_f=0.58, followed by rechromatography with EtOAc–hexane (45+55), Vis. λ_{max} nm (455), 480, (505). ^1H NMR ($CDCl_3$) δ 1.98 (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, $J_{ax,ax}$=14 Hz, $J_{ax,eq}$=6Hz, H-3); 5.80 (m, $J_{3',2'}$=7 Hz, $J_{3',4'}$=16 Hz, H-3′ end-of-chain olefinic proton). MS *m/z* 582 (M^+, 52 %), M−18 (9 %), M−58 (7 %), M−92 (5 %), M−106 (100 %).

Reduced flexixanthin (*3*). *1a* in MeOH was treated with $NaBH_4$. TLC on Kieselgel developed with Me_2CO–hexane (40+60) gave *3* in virtually quantitative yield, Vis. λ_{max} nm 449, 473, 504.

Reduced flexixanthin diacetate (*3b*). *3* Was acetylated and the reaction mixture was worked up in the usual manner. TLC on Kieselgel with Me_2CO–hexane (40+60) gave two products with identical Vis. and MS; R_f=0.38 (minor), 0.43 (major); presumably *cis* and *trans* C-3,4 diesters. Vis λ_{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.[15]

2′-Hydroxyflexixanthin (*5a*). R_f=0.48 Kieselgel Me_2CO–hexane (40+60). Vis. λ_{max} nm (454), 480, 505. ^1H NMR ($CDCl_3$) 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 $J_{3',2'}$=7 Hz, $J_{3',4'}$=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′-hydroxyflexixanthin (*6*). *6* Was obtained by $NaBH_4$ reduction of *5a*. Vis. λ_{max} nm

447, 473, 504.

Reduced 2'-hydroxyflexixanthin triacetate (*6b*). Acetylation of *6* provided *6b*. Vis. λ_{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'Hydroxyflexixanthin (*5a*) *dicamphanate*. The camphanate was prepared from (−)-camphanoyl chloride by the general[7] procedure. HPLC (Perkin-Elmer Series 2LC equipped with LC-85 spectrophotometric detector at 491 nm) on Spheri-5 silica, 5 μm[16] (25×0.46 cm) column; eluted with EtOAc-hexane (35+65), flow 2 ml/min, showed one peak R_T=7.68′ with a small inflexion at R_T=6.88′ under conditions where the diastereomeric astaxanthin camphanates were well separated. On Cyano Spheri-5 column[16] (25×0.46 cm) eluted with hexane-*iso*propyl acetate−Me_2CO (72+16+12), flow 2 ml/min, R_T=8.83′ (2 %) and R_T=10.15′ (98 %) under conditions where the all-*trans* diastereomeric astaxanthin camphanates had R_T 6.96′, 8.32′ and 10.15′ with baseline separation.

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