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Bacterial Carotenoids XL.* 2′-Hydroxyflexixanthin

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The carotenoids of the gliding flexibacteria have been the subject of a number of investigations.[1-6] Flexixanthin (*2*) and the less abundant deoxyflexixanthin (*1*) are peculiar to several flexibacteria;[1-3] other flexibacteria produce structurally related carotenoids.[1,2,4-6]

We now report the isolation of flexixanthin (*2*) and the previously undescribed 2′-hydroxyflexixanthin (*3*) from a non-gliding bacterium, strain NIVA BR6-64, of uncertain taxonomic position.

Table 1. Properties of the carotenoids studied.

Carotenoid	λ_{max} in acetone in nm	R_F-values a	b
Deoxyflexixanthin (*1*)	478	0.52	0.55
Flexixanthin (*2*)	478,503	0.40	0.27
2′-Hydroxyflexixanthin (*3*)	478,504	0.19	0.10
Flexixanthin acetate (*4*)	478	0.50	0.48
4 Trimethylsilyl ether (*5*)	477	0.68	0.90
Dehydroflexixanthin (*6*)	478	0.38	0.0
2′-Ketoflexixanthin (*7*)	500	0.16	0.18
2′-Ketoflexixanthin acetate (*8*)	499	0.31	
8 Trimethylsilyl ether (*9*)	500	0.58	0.87
2′-Ketodehydroflexixanthin trimethylsilyl ether (*10*)	499	0.40	0.0

a. Schleicher & Schüll No. 287 (kieselguhr) paper; 10 % acetone in petroleum ether.
b. Schleicher & Schüll No. 288 (aluminium oxide) paper; 20 % acetone in petroleum ether.

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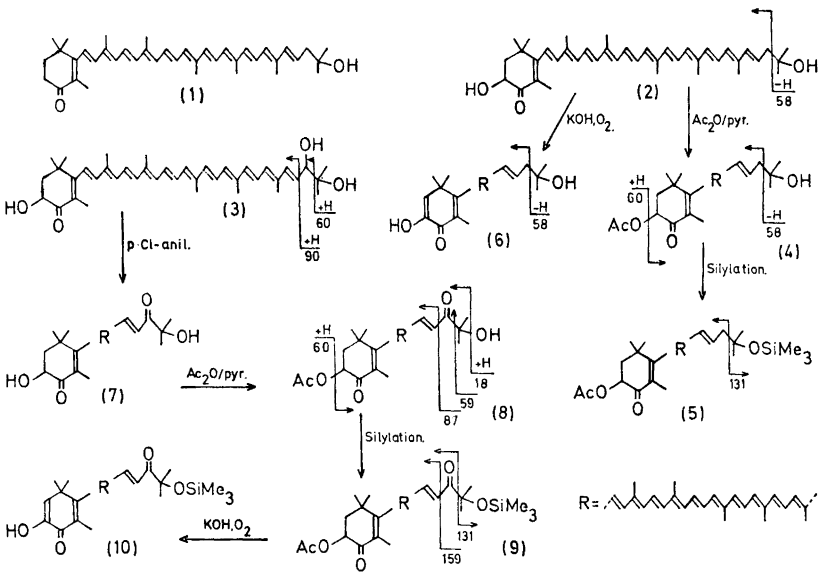

Scheme 1.

The carotenoids (14 mg from 110 l of culture, 331 g wet cells) from the acetone extracted cell residue consisted of a minor pigment (0.4 % of the total carotenoids) tentatively identified as deoxyflexixanthin (*1*), flexixanthin (*2*, 69 %) and 2′-hydroxyflexixanthin (*3*, 30 %).

The identification of deoxyflexixanthin (*1*) was based on its electronic spectrum and chromatographic behaviour (Table 1).

Flexixanthin (*2*) was identified from electronic and mass spectra; the formation of flexixanthin acetate (*4*) on acetylation and from the monotrimethylsilyl ether *5* formed by silylation of *4*; as well as on the transformation of *2* to dehydroflexixanthin (*6*) on alkali treatment (Scheme 1, also including important mass-spectrometric fragmentations and Table 1); *cf.* Refs. 3, 7. The derivative *4* could not be chromatographically separated from the corresponding derivative of authentic [3] flexixanthin.

2′-Hydroxyflexixanthin (*3*) exhibited an electronic spectrum in visible light identical with that of flexixanthin (*2*). Its mass spectrum showed the molecular ion peak at *m/e* 598 (consistent with the formula $C_{40}H_{54}O_4$), confirmed by the common losses of 92 and 106 mass units.[8] Diagnostically important peaks were observed at M − 18(H_2O), M − 60, and M − 90; the two latter ions are characteristic [9] of the acyclic end of plectaniaxanthin (3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-1′,2′-diol[10]). The chromatographic behaviour (Table 1), electronic and mass spectra can all be accomodated by structure *3*. Supporting evidence was obtained from the chemical transformations summarized in Scheme 1. Oxidation of *3* with *p*-chloranil [11] gave a single product *7*. The bathochromic shift [12] and decrease in polarity relative to *3* supported the presence of an allylic hydroxy group in 2′-position in *3*. Acetylation of the diketone *7* gave the acetate *8* with higher R_F value and unchanged electronic spectrum. The mass spectrum of *8* had the molecular ion at *m/e* 638, confirming the formation of a monoacetate and thus demonstrating the presence of a second secondary or primary hydroxy group in *3*. In addition to the common M − 92 and M − 106 ions, fragment ions of *8* were noted at M − 60 (acetic acid) and the expected M − 59 and M − 87 ions associated with cleavages of the bonds in positions α to the 2′-keto group. Prominent M − 16 [9] and M − 18 ions were also observed. The diketo-monoacetate (*8*) provided a trimethylsilyl ether *9* on silylation [7] with the expected polarity (Table 1).

The mass spectrum of *9* showed the molecular ion at *m/e* 710 (consistent with the formula $C_{45}H_{62}O_5Si$) and diagnostically useful peaks at M − 60 (acetic acid), M − 131 ($C_6H_{15}OSi$) and *m/e* 131. Finally the α-ketol arrangement of *3* was demonstrated by weak alkali treatment of *9* on the micro scale leading to the acidic diosphenol *10*, completely retained on alumina paper; hydrolysis of the tertiary silyl ether group is expected to require stronger conditions.[7] By the new nomenclature[10] 2′-hydroxy-flexixanthin is 3,1′,2′-trihydroxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-4-one.

The carotenoid analysis may indicate a relationship between the present non-gliding bacterium and the gliding flexibacteria. Whereas nutritional and physiological studies by Ormerod and Kristensen[13] have not provided information facilitating further classification, the % GC ratio falls within the range characteristic of *Flexibacteriales*.[13,14] However, although flexixanthin (*2*) has not yet been encountered outside flexibacteria it should be mentioned that carotenoids structurally related to *2* and *3* are reported from *Mycobacterium phlei* strain Vera[12] (the aglycone of 4-ketophleixanthophyll), from species of *Myxobacteriales: Sorangium compositum* (the aglycone of myxobacton ester)[15] and *Stigmatella aurantiaca*.[16] These carotenoids differ from *2* and *3* by lacking the α-ketol arrangement of the β-ring.

Experimental. Strain NIVA BR6-64 from the collection of the Norwegian Institute of Water Research was obtained from Docent J. G. Ormerod, Botanical Institute, University of Oslo. The cultivation[17] was carried out in a pilot plant fermentor in a trypton-sucrose medium.

Methods and instruments commonly used in this laboratory were employed.[18,19] The carotenoids were extracted with acetone, saponification being avoided in the isolation procedure, and separated by chromatography on preparative silica gel G plates using 30 % acetone in petroleum ether.

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