THE IDENTIFICATION OF DESMETHYLSPHEROIDENONE AS A MAJOR CAROTENOID IN AEROBIC CULTURES OF *RHODOPSEUDOMONAS CAPSULATA*

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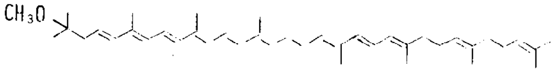
1. Introduction

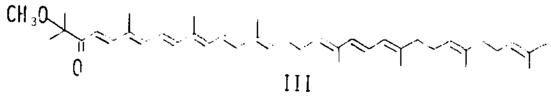
Purple non-sulphur photosynthetic bacteria of the Rhodospirillaceae characteristically contain acyclic carotenoids with tertiary methoxy substituents at C-1. For example, in most *Rhodopseudomonas* species, including *Rps. sphaeroides*, *Rps. gelatinosa* and *Rps. capsulata*, grown in the light under anaerobic conditions the main carotenoids are spheroidene (1-methoxy-3,4-didehydro-1,2,7′,8′-tetrahydro-ψ,ψ-carotene, I) and 'hydroxyspheroidene' (1′-methoxy-3′,4′-didehydro-1,2,7,8,1′,2′-hexahydro-ψ,ψ-caroten-1-ol, II). In the presence of oxygen these compounds are rapidly converted into their 2-oxo derivatives, spheroidenone (1-methoxy-3,4-didehydro-1,2,7′,8′-tetrahydro-ψ,ψ-caroten-2-one, III) and 'hydroxyspheroidenone' (1′-hydroxy-1-methoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-caroten-2-one, IV).

When *Rps. capsulata* was cultured aerobically in the dark, the total carotenoid content was much lower than in phototrophically grown cells, and one of the main carotenoids was found to be similar, but not identical, to hydroxyspheroidenone. This unusual carotenoid has now been characterized by MS and ^{1}H NMR spectroscopy as the novel 'desmethylspheroidenone' (1-hydroxy-3,4-didehydro-1,2,7′,8′-tetrahydro-ψ,ψ-caroten-2-one, V). Desmethylspheroidene and desmethylspheroidenone have also been detected in cultures of *Rps. capsulata* grown in the light.

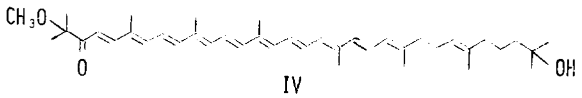


I

II



III



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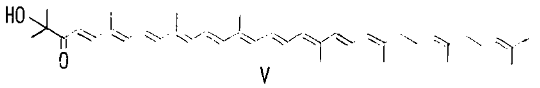

V

2. Methods

Rhodopseudomonas capsulata (wild-type strain St Louis, ATCC 23782) was grown in darkness, with aeration, in the medium of [1]. Cells were harvested by centrifugation and the pigments extracted and separated by standard procedures [2]. The final purification was effected by thin-layer chromatography on silica gel G with diethylether–light petroleum (b.p. 40–60°C) (1:1, v/v) as developing

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solvent, followed by filtration, in diethyl ether, through a small column of neutral alumina (Brockmann grade III).

Reduction with sodium borohydride, and attempted acetylation were performed by standard procedures [2].

Mass spectra were determined by Mr M. Prescott, using an AEI MS12 instrument with the direct insertion probe, ion source temp. 200°C and ionizing voltage 70 eV.

^{1}H NMR (Fourier transform) spectra of samples in $CDCl_3$ were obtained with a Varian XL-100 instrument.

3. Results and discussion

One of the main carotenoids isolated from dark, aerobic cultures of *Rhodopseudomonas capsulata* had an absorption spectrum (λ_{max} in ethanol at ~487 nm) similar to those of spheroidenone and hydroxyspheroidenone. Like the latter two compounds, the new carotenoid could not be acetylated, and was reduced by sodium borohydride to a product with λ_{max} at 428, 453, 484 nm. Chromatography showed the new carotenoid to be slightly less polar than hydroxyspheroidenone.

The mass spectrum was entirely consistent with the structure desmethylspheroidenone (1-hydroxy-3,4-didehydro-1,2,7',8'-tetrahydro-ψ,ψ-caroten-2-one). The parent ion $M^{+\cdot}$ was at *m/e* 568 (90%, $C_{40}H_{56}O_2$) and major fragment ions were present at *m/e* 476 (10%, M-92) and 462 (100%, M-106) due to losses of toluene and *m*-xylene, characteristic of carotenoids. The presence of the acyclic 7',8'-dihydro end-group was indicated by strong fragment ions at *m/e* 431 (10%) and 325 (25%) due to losses of 137 mass units from the $M^{+\cdot}$ and $(M\text{-}106)^{+}$ ions by cleavage of the 'bis-allylic' C-7',8' bond. The 1-hydroxy-2-oxo-3,4-didehydro end-group was indicated by the presence of characteristic fragment ions at *m/e* 552 (2%, M-16), 550 (3%, M-18), 509 (2%, M-59), 480 (12%, M-88) and 59 (100%) [3]. The absence of methoxy-groups was shown by the absence of losses of 32 and 73 mass units in the mass spectrum.

The structural assignment was confirmed by the ^{1}H NMR spectrum. The 'in-chain' methyl groups at C-5,9,13,13' gave signals at δ 1.97–2.00 ppm. The 1,1-methyl substituents of the 1-hydroxy-2-oxo end-group gave signals at 1.41 ppm, whereas the 1',1'-methyl groups gave signals at 1.58 (C-17') and 1.65 ppm (C-16') and the C-5' and C-9' methyl group signals were observed at 1.58 ppm and 1.79 ppm, respectively. These values are in agreement with those tabulated in [3] for these carotenoid end-groups.

The structure of the *Rps. capsulata* pigment is thus confirmed as desmethylspheroidenone, a new carotenoid. Although the 1-hydroxy-1,2-dihydro-2-oxo-3,4-didehydro end-group has been found in some bacterial carotenoids [4,5] this is the first report of any carotenoid in photosynthetic bacteria containing a 2-oxo group but with the C-1 hydroxy substituent unmethylated.

Desmethylspheroidenone and desmethylspheroidene were also detected in cultures grown completely in light conditions. It would therefore be of considerable interest to determine the fate of the desmethylspheroidenone when dark, aerobic cultures of *Rps. capsulata* are transferred to light, aerobic or anaerobic conditions, and to examine the possible role of this compound in the development of the photosynthetic apparatus, especially since preliminary experiments have shown that the behaviour of desmethylspheroidenone is quite different from that of spheroidene and spheroidenone when dark-grown cultures are illuminated [4,6].

Finally a warning must be given about the identification of some of the carotenoids of photosynthetic bacteria. The light absorption spectroscopic properties of desmethylspheroidenone and hydroxyspheroidenone are identical, and the chromatographic properties of the two compounds differ only slightly – the difference only becomes at all clear when the two samples are compared directly. Casual identifications of hydroxyspheroidenone and related carotenoids by their light absorption spectra and chromatographic behaviour should therefore only be made with the greatest care, and confirmation of the identification by mass spectrometry is strongly to be recommended.

Acknowledgements

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