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SHORT COMMUNICATION

THE IDENTIFICATION OF SPHEROIDENE AND HYDROXYSPHEROIDENE IN DIPHENYLAMINE-INHIBITED CULTURES OF *RHODOSPIRILLUM RUBRUM*

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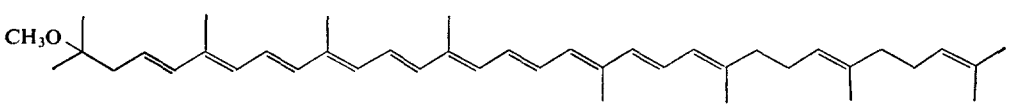
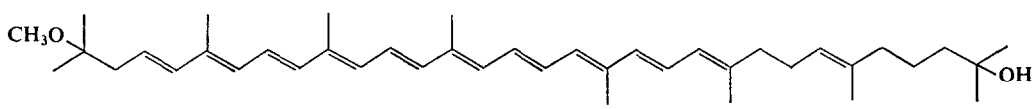
Abstract—Two minor carotenoids isolated from the photosynthetic bacterium *Rhodospirillum rubrum*, grown anaerobically in the presence of diphenylamine, have been identified as spheroidene and hydroxyspheroidene by comparison (co-chromatography, visible absorption spectra and mass spectra) with samples of these pigments isolated from *Rhodopseudomonas spheroides*. These compounds, which have not previously been reported in *Rhodospirillum rubrum*, are not present in cultures grown without diphenylamine.

INTRODUCTION

PHOTOSYNTHETIC bacteria of the Athiorhodaceae synthesize a range of acyclic carotenoids with tertiary hydroxy- and methoxy-substituents at C-1 and C-1′ (carotene numbering). *Rhodopseudomonas* species accumulate 2-oxo-carotenoids, e.g. spheroidenone,[1,2] which are thought to be biosynthesized from neurosporene, via spheroidene (pigment Y) (I) and hydroxyspheroidene (hydroxy-Y) (II).[3,4]



(I)



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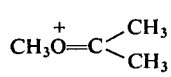

(III)

[1] C. B. VAN NIEL, *Anthonie van Leeuwenhoek, J. Microbiol. Serol.* **12**, 156 (1947).
[2] T. W. GOODWIN, D. G. LAND and H. G. OSMAN, *Biochem. J.* **59**, 491 (1955).
[3] S. L. JENSEN, G. COHEN-BAZIRE and R. Y. STANIER, *Nature* **192**, 1168 (1961).
[4] K. V. SUNADA and R. Y. STANIER, *Biochim. Biophys. Acta* **107**, 38 (1965).

Other species, e.g. *Rhodospirillum rubrum*, do not synthesize oxo-derivatives, but accumulate large amounts of spirilloxanthin.[5] When *R. rubrum* is grown in the presence of diphenylamine (DPA), spirilloxanthin synthesis is inhibited and a range of more saturated carotenoids can be isolated. From studies of the pigments found in such DPA-inhibited cultures, Jensen *et al.*[3,6] postulated a scheme for the biosynthesis of spirilloxanthin from lycopene, rather than via spheroidene and hydroxyspheroidene, since no members of the spheroidene–spheroidenone series were found in *R. rubrum*, with or without DPA present. Later work[7] has shown that *Rhodopseudomonas gelatinosa* produces both the spirilloxanthin and spheroidenone series, and it was suggested that spirilloxanthin could arise from spheroidene and hydroxyspheroidene in this organism. We now report that DPA-inhibited *Rhodospirillum rubrum* is also able to synthesize spheroidene and hydroxyspheroidene.

RESULTS AND DISCUSSION

As part of a detailed re-analysis of the carotenoids of *Rhodospirillum rubrum*, grown anaerobically in the presence of DPA, two compounds, *A* and *B* were isolated, which had similar visible absorption spectra, but different chromatographic properties. The visible spectra of these compounds were reminiscent of the spectra of spheroidene and hydroxyspheroidene, authentic specimens of which were therefore isolated from *Rhodopseudomonas spheroides* and compared with the unknown pigments. In a number of different thin-layer chromatography systems, the less polar pigment, *A*, was found to co-chromatograph with spheroidene, while the more polar pigment, *B*, was not separated from hydroxyspheroidene.

The visible absorption spectra of pigment *A* and spheroidene were identical [$\lambda_{max.(petrol)}$ at 428, 454, 485 nm] as were the spectra of pigment *B* and hydroxyspheroidene [$\lambda_{max.(petrol)}$ at 427, 453, 484 nm].

The mass spectra of pigment *A* and spheroidene were identical, showing a molecular ion at *m/e* 568 (accurate determination 568·4622, 568·4627 respectively, $\equiv C_{41}H_{60}O$). Other major peaks were obtained at *m/e* 536 (M - 32; [—CH_3OH]), *m/e* 495 (M - 73), *m/e* 476 (M - 92),[8] *m/e* 462 (M - 106)[8] and *m/e* 389 (M - 106 - 73). A very strong peak was obtained for *m/e* 73 (accurate determination for spheroidene, 73·0644 $\equiv C_4H_9O$ (III)). Metastable ions were observed in each case at *m/e* 327·5. The ratio of the (M - 92)/(M - 106) peaks was approximately 0·25 in each case, in line with the values reported for similar compounds.[8]

Similarly, the mass spectra of pigment *B* and hydroxyspheroidene were identical, showing a molecular ion at *m/e* 586 (accurate determination for pigment *B*, 586·4777 $\equiv C_{41}H_{62}O_2$). Other major peaks were obtained at *m/e* 568 (M - 18; [—H_2O]), *m/e* 554 (M - 32; [—CH_3OH]), *m/e* 536 (M - 18 - 32), *m/e* 513 (M - 73), *m/e* 494 (M - 92), *m/e* 480 (M - 106) and *m/e* 407 (M - 106 - 73). A very strong peak was again observed at *m/e* 73 (III). Metastable ions were observed in each case at *m/e* 345·1. The ratio of the (M - 92)/(M - 106) peaks in each case was close to 0·25.

This work therefore proves that the two pigments isolated from DPA-inhibited cultures of *Rhodospirillum rubrum* are spheroidene and hydroxyspheroidene. These pigments were present when the bacterium was grown anaerobically in the presence of DPA, but were not observed when it was grown anaerobically without DPA. It is likely that the pigment

[5] C. B. VAN NIEL and J. H. C. SMITH, *Arch. Mikrobiol.* **6**, 219 (1935).
[6] S. L JENSEN, G. COHEN-BAZIRE, T. O M. NAKAYAMA and R. Y. STANIER, *Biochim. Biophys. Acta* **29**, 477 (1958).
[7] K. E. EIMHJELLEN and S. L. JENSEN, *Biochim. Biophys. Acta* **82**, 21 (1964).
[8] C. R. ENZELL, G. W FRANCIS and S. L JENSEN, *Acta Chem. Scand.* **22**, 1054 (1968).

previously observed, but not identified, by Goodwin and Osman[9] ("compound G") and by Jensen *et al.*[6] ("P450") was spheroidene.

The possible biosynthetic implications of the presence of these and other new compounds will be discussed in a forthcoming paper.

EXPERIMENTAL

Cultures and Cultural Conditions

Cultures of *Rhodospirillum rubrum* (NCIB 9086) were obtained from the Science Research Council, National Collection of Industrial Bacteria, Aberdeen, Scotland. The bacterium was grown anaerobically in glass-stoppered roux bottles in the light for 6–8 days at 30 ± 2°, on the malate–glutamate medium of Lascelles.[10] The DPA concentration in the medium was $6{\cdot}9 \times 10^{-5}$ M.

A culture of *Rhodopseudomonas spheroides* (NCIB 8253) was kindly donated by Dr. N. G. Carr, and the organism was grown in glass-stoppered roux bottles anaerobically in the light for 5 days at 30 ± 2°, on the malate–glutamate medium of Lascelles.

Pigment Extraction and Purification [(*a*) Rhodospirillum rubrum]

Cells were harvested by centrifugation and the pigments extracted with acetone in the normal way. The extract was chromatographed on a column of alumina (Brockmann Grade III), which was developed with increasing amounts of diethyl ether (E) in light petroleum (P). The fractions eluted with 1–40% E/P were combined and evaporated, and further separated by TLC on Silica gel G with 40% E/P as developing solvent. This gave four main broad bands containing, in order of increasing polarity, monomethoxy-, dimethoxy-, monohydroxy- and monohydroxymonomethoxy-compounds respectively. The individual compounds in the monomethoxy-group were separated by TLC on MgO–Kieselgur G (1:1), with 10% acetone (A)/P as developing solvent. Pigment *A* was eluted and further purified by TLC on MgO–Kieselgur G (1:1) with 30% A/P, and TLC on silica gel G with 10% E/P as developing solvent. Pigment *B* was obtained by TLC of the monohydroxymonomethoxy-fraction on MgO–Kieselgur G (1:1) with 30% A/P as developing solvent, and further purified by TLC on MgO-Kieselgur G (1:1) with 50% A/P, and TLC on Silica gel G with 30% E/P as developing solvent.

Spheroidene and hydroxyspheroidene were extracted from *Rhodopseudomonas spheroides* and purified in a similar way.

Co-chromatography

Pigment *A* and spheroidene, and pigment *B* and hydroxyspheroidene were co-chromatographed on the following TLC systems:

(a) Silica gel G with (i) 5% E/P, (ii) 10% E/P, (iii) 30% E/P.
(b) Alumina G with (i) 5% E/P, (ii) 10% E/P, (iii) 30% E/P.
(c) MgO–Kieselgur G with (i) 20% A/P, (ii) 40% A/P.

Mass Spectrometry

Mass spectra were kindly determined by I.C.I. (Pharmaceuticals) Ltd., through the good offices of Dr. G. A. Snow.

The mass spectra were obtained on an A.E.I. MS 9 instrument, using the direct inlet system, and an ion source temperature of 280°.

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[9] T. W. Goodwin and H. G. Osman, *Biochem. J.* **56**, 222 (1954).
[10] J. Lascelles, *Biochem. J.* **62**, 78 (1956).