THE OCCURRENCE OF HYDROXY-DERIVATIVES OF PHYTOENE AND PHYTOFLUENE IN DIPHENYLAMINE-INHIBITED CULTURES OF *RHODOSPIRILLUM RUBRUM*

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1. Introduction

Photosynthetic bacteria of *Athiorhodaceae* synthesize a range of acyclic carotenoids with tertiary hydroxy- and methoxy-substituents at C-1 and C-1′ (carotenoid numbering). *Rhodopseudomonas* species accumulate 2-oxo-carotenoids, e.g. spheroidenone [1, 2] which are thought to be synthesized via neurosporene. Other genera, e.g. *Rhodospirillum rubrum*, do not synthesize oxo-derivatives, but accumulate large amounts of spirilloxanthin [3]. 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 DPA-inhibited cultures, Jensen et al. [4, 5] postulated a scheme for the biosynthesis of spirilloxanthin from lycopene. Later work [6, 7, 8] has suggested that alternative pathways of spirilloxanthin biosynthesis may operate in *R. rubrum*. We now report the isolation and characterization of 1-hydroxy-1,2-dihydrophytoene, and 1-hydroxy-1,2-dihydrophytofluene from *R. rubrum*, and the detection of traces of the corresponding 1-methoxy-derivatives. This organism is capable of hydroxylation and methoxylation of the early carotenoid precursors, phytoene and phytofluene, under conditions of diphenylamine inhibition.

2. Experimental procedures

Cultures of *Rhodospirillum rubrum* (NCIB 9086) were obtained from the Science Research Council, National Collection of Industrial Bacteria, Aberdeen, Scotland, and were grown anaerobically in the light in the presence of diphenylamine, as previously described [8]. The cells were harvested, pigments extracted and monohydroxy-fraction obtained as previously described. The monohydroxy-compounds were separated by thin-layer chromatography (TLC) on MgO-Kieselgur G (1:1) with 1% (v/v) acetone in light petroleum as developing solvent. The hydroxyphytofluene band, recognised by its green fluorescence under UV light, was further purified by TLC on MgO-Kieselgur G (1:1) with 1% (v/v) acetone in light petroleum, and on Silica gel G with 25% diethyl ether in light petroleum as developing solvent. No separation of hydroxyphytoene and hydroxyphytofluene was achieved on thin layers of Silica gel G, MgO-Kieselgur G, almunina G, or $ZnCO_3$-Kieselgur G (1:1).

Thrimethylsilyl ethers were prepared from the mixture by the method of McCormick and Jensen [9], separated by TLC on MgO-Kieselgur G (1:1) with 1% acetone in light petroleum as developing solvent, and finally purified by TLC on Silica gel G with 2% diethyl ether in light petroleum as developing solvent.

Electronic spectra were determined, in light petroleum, in a Unicam SP 800 recording spectrophotometer.

Mass spectra were kindly determined by Mrs. A.M.Ball on an A.E.I. MS 12 instrument.

3. Results and discussion

The presence of a phytofluene derivative in the

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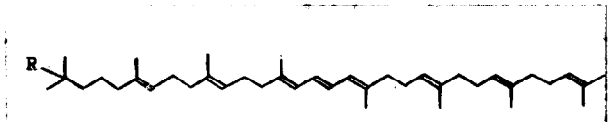
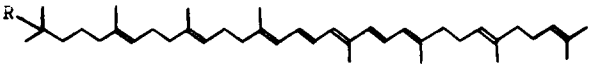
monohydroxy-fraction of the unsaponifiable material from diphenylamine-inhibited cultures of *Rhodospirillum rubrum* was revealed by the strong greenish fluorescence under UV light. The UV spectrum of this compound, however, showed absorption maxima at 275, 285, 298 nm (characteristic of phytoene and its derivatives) in addition to the absorption maxima at 331, 348, 367 nm, characteristic of phytofluene and its derivatives. All attempts to separate the phytoene derivative from the phytofluene derivative were unsuccessful. The compounds did not form acetates (i.e. were not primary or secondary alcohols) but trimethylsilyl ethers were prepared, indicating the presence of tertiary hydroxyl groups. The trimethylsilylethers were separated and purified by TLC, and their mass spectra determined.

The phytoene derivative gave a parent ion M^+ at *m/e* 634 ($C_{43}H_{74}OSi$) and major fragment ions were observed at *m/e* 544 (M-$(CH_3)_3SiOH$), and at *m/e* 429 (M-205) and 339 (M-295) caused by the characteristic cleavage of the bisallylic bonds adjacent to the triene chromophore. These fragmentations were supported by the presence of metastable peaks. The mass spectrum and other data are consistent with structure Ia for the trimethylsilyl ether, and Ib (1-hydroxy-1,2-dihydrophytoene) for the naturally occurring compound.

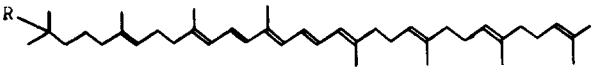


Ia (R = -OSi$(CH_3)_3$)
Ib (R = -OH)

The mass spectrum of the phytofluene derivative showed the parent ion M^+ at *m/e* 632 ($C_{43}H_{72}OSi$), and fragment ions were obtained at *m/e* 542 (M-$(CH_3)_3$ SiOH), 495 (M-137), 427 (weak, M-205), 405 (M-227, or M-90-137), 337 (M-295). The mass spectrum data confirm the structure of this compound as the trimethylsilyl ether of 1-hydroxy-1,2-dihydrophytofluene; the relative intensities of the fragment ions suggest structure IIa for the trimethylsilyl ether and structure IIb for the natural compound, rather than the alternative structure IIIa and IIIb. However, such small quantities of this compound were available that only a weak mass spectrum was obtained, and the metastable ions which would have allowed a distinction between the two possible structures to be made were not detected.



IIa (R = -OSi$(CH_3)_3$)
IIb (R = -OH)



IIIa (R = -OSi$(CH_3)_3$)
IIIb (R = -OH)

The presence, of a compound with the properties of a hydroxyphytofluene in DPA-inhibited cultures of *R. rubrum*, has been reported by other workers [5, 10, 11]. This is the first report of natural occurrence of a hydroxy-derivative of phytoene in photosynthetic bacteria.

Examination of the monomethoxy-fraction from DPA-inhibited cultures of *R. rubrum* has revealed the presence of two compounds tentatively identified from their chromatographic properties and electronic spectra as methoxy-derivatives of phytoene and phytofluene. The quantities isolated, however, were too small to allow further characterisation.

The presence of these hydroxy- and methoxy-derivatives of phytoene and phytofluene in DPA-inhibited cultures of *R. rubrum* shows that this organism is capable of hydroxylation and methoxylation of the early carotenoid precursors, phytoene and phytofluene, but does not necessarily imply that these compounds are intermediates in the synthesis of the more unsaturated carotenoids such as spirilloxanthin under normal or abnormal conditions. The possible importance of the presence of these compounds in relation to the biosynthesis of carotenoids in the *Athiorhodaceae* will be considered elsewhere.

Acknowledgements

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References

[1] C.B.van Niel and A.van Leeuwenhoek, J. Microbiol. Serol. 12 (1947) 156.
[2] T.W.Goodwin, D.G.Land and H.G.Osman, Biochem. J. 59 (1955) 491.
[3] C.B.van Niel and J.H.C.Smith, Arch. Mikrobiol. 6 (1935) 219.
[4] S.L.Jensen, G.Cohen-Bazire and R.Y.Stanier, Nature 192 (1961) 1168.
[5] S.L.Jensen, G.Cohen-Bazire, T.O.M.Nakayama and R.Y. Stanier, Biochim. Biophys. Acta 29 (1958) 477.
[6] B.H.Davies and E.A.Holmes, Biochem. J. 113 (1969) 34P.
[7] B.H.Davies, E.A.Holmes, D.E.Loeber, T.P.Toube and B.C.L.Weedon, J. Chem. Soc. C. (1969) 1266.
[8] H.C.Malhotra, G.Britton and T.W.Goodwin, Phytochemistry 8 (1969) 1047.
[9] A.McCormick and S.L.Jensen, Acta Chem. Scand. 20 (1966) 1989.
[10] T.W.Goodwin and H.G.Osman, Biochem. J. 56 (1954) 222.
[11] B.H.Davies, in: Natural substances formed biologically from mevalonic acid, Biochemical Society Symposium communication, Liverpool, April 1969.