Bacterial Carotenoids

VIII. * A Structural Scheme for the Biosynthesis of Coloured Carotenoids in the Photosynthetic Bacterium *Rhodospirillum rubrum*
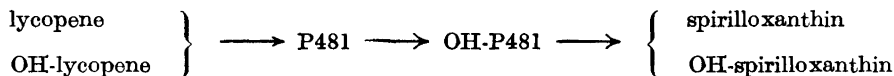
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In the present paper the chemical structures of some bacterial carotenoids, already discussed in a series of papers (Bacterial Carotenoids I—VII) in this journal [1-7], are correlated with the results of biosynthetic studies.

By kinetic studies of the carotenoid biosynthesis in the photosynthetic purple bacterium *Rsp. rubrum* performed by Stanier and his associates [8], it was demonstrated that spirilloxanthin was formed at the expense of the lycopene group (lycopene and OH-lycopene) and the P481 group (P481 and OH-P481) in a manner strongly suggesting the sequence:

$$\left.\begin{array}{c} \text{lycopene} \\ \text{OH-lycopene} \end{array}\right\} \longrightarrow \text{P481} \longrightarrow \text{OH-P481} \longrightarrow \left\{\begin{array}{c} \text{spirilloxanthin} \\ \text{OH-spirilloxanthin} \end{array}\right.$$

When the present work was started the following structures for three of these carotenoids, namely for lycopene (I), OH-lycopene assumed to be identical with lycoxanthin (II)[9,10], and spirilloxanthin (III)[11,12] were largely accepted.

The transformation from lycopene (I) to spirilloxanthin (III) through a reasonable number of simple chemical reactions *via* lycoxanthin (II) and the unknown components P481 and OH-P481 is not easily envisaged. The aim of the present investigation therefore was to check the correctness of the previously assigned structures and to establish the structures of the unknown components of this series. Recent work has led to the establishment of chemical structures for spirilloxanthin (IV)[2,13], monodemethylated spirilloxanthin (OH-spirilloxanthin) (V)[7], rhodovibrin (OH-
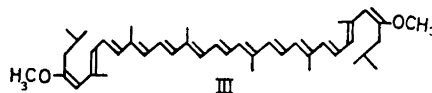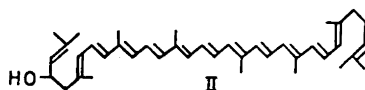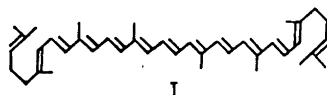
P481) (VI or VII)[1,5] and rhodopin (OH-lycopene) (VIII)[3,4]. The available data for P481[9,1,8,14] indicate the structures (IX) or (X) for this carotenoid. From the formal relationship between rhodovibrin (VI or VII) and P481 (IX or X), the denomination anhydro-rhodovibrin is suggested for P481. Additional evidence for the identity of lycopene from *Rsp. rubrum* and authentic lycopene (I) has been provided by a quantitative comparative study of the iodine catalyzed equilibrium mixtures of the two carotenoids [14].

A structural interpretation of the biochemical reactions leading from lycopene to spirilloxanthin has been attempted by Barber, Jackman and Weedon [13]. Some of the structures favoured by these authors, however, were later shown to be incorrect [3-5].

The establishment of chemical structures for the carotenoids participating in these transformations, makes possible a more definite interpretation in chemical terms of the reactions involved in later steps of carotenoid biosynthesis in *Rsp. rubrum*.

From the chemical structure of rhodopin (VIII) compared to that of lycopene (I) and anhydro-rhodovibrin (IX or X), the position of rhodopin in the biosynthetical sequence can be settled with reasonable assurance. A similar inference can be made for monodemethylated spirilloxanthin (V), which is obviously an intermediate between rhodovibrin (VI or VII) and spirilloxanthin

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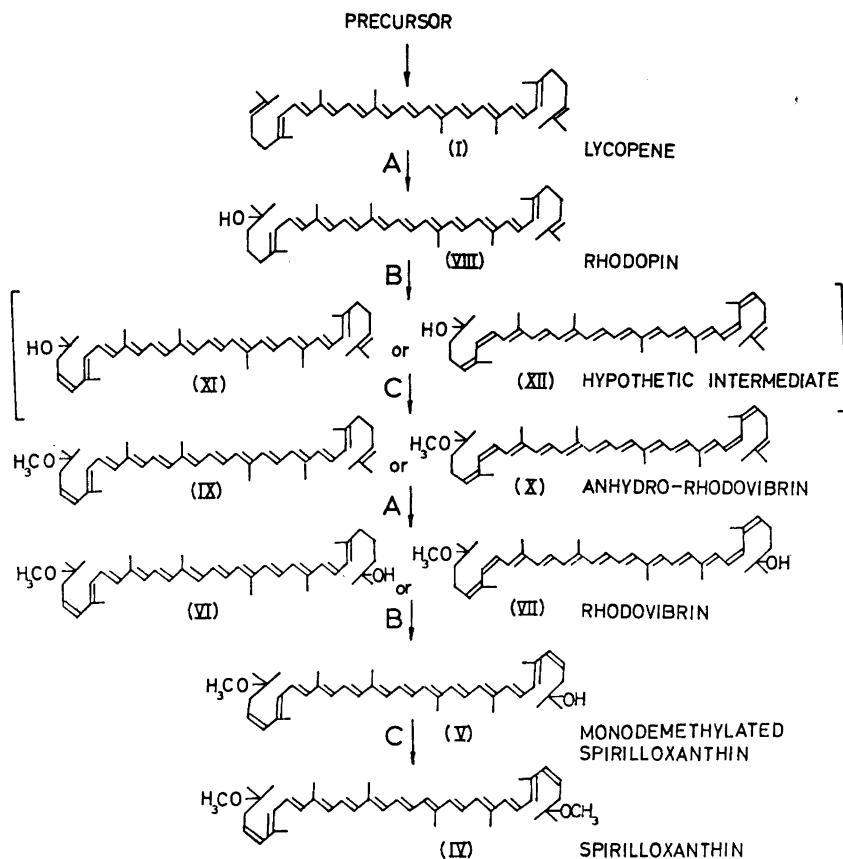
(IV). The final biosynthetic sequence can then be represented in the manner given above.

The intermediate (XI or XII) is hypothetic, and has not been isolated.

Based on the scheme depicted above the biochemical events leading from lycopene to spirilloxanthin can be envisaged in terms of three simple reactions, the sequence of which reflects the relative rates with which they proceed:

A. Addition of H_2O to an *iso*propylidene double bond with the formation of a tertiary alcohol.

B. Introduction of an additional double bond either in conjugation with the double bond chain at the same side as the hydroxyl group, or as an a,ω-dehydrogenation.

C. Methylation of the tertiary hydroxyl group.

To explain the formation of rhodopin, it is assumed that reaction A is faster than reaction B. The kinetic evidence [8] further reveals that the reactions B and C proceed with very similar rates. Thus neither products of the type (XI or XII), expected as intermediates if reaction B were faster than reaction C, nor a methylated rhodopin (XIII) or methylated rhodovibrin (XIV or XV), predicted as intermediates in the opposite case, have been isolated. Moreover, monodemethylated spirilloxanthin (V) has been isolated only in very small amounts.

The compounds (XI or XII), (XIII), and (XIV or XV) would, however, in the non-

XIII

XIV

XV
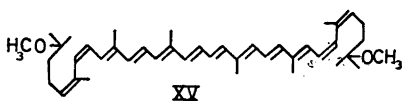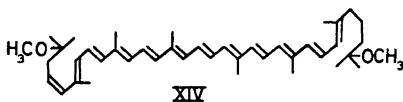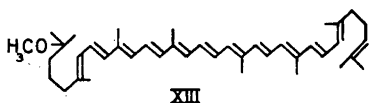
crystalline state be difficult to distinguish from rhodovibrin, lycopene and anhydro-rhodovibrin respectively. The given sequence of the successive steps B and C is based on the isolation of monodemethylated spirilloxanthin. The suggestion that the methylation reaction C, which is dependent on the availability of intracellular methyl donors is slower than the oxidation reaction B, seems also the more plausible.

Biological hydroxylation mechanisms have been discussed by Hunt, Hughes and Lowenstein [15] and by Massart and Vercauteren [16]. Analogies for the net reaction (addition of H_2O to a carbon-carbon double bond) involved in the hydroxylation of lycopene and anhydro-rhodovibrin can be found among the common metabolites such as the transformation of fumaric to malic acid and the conversion of cis-aconitic acid to citric acid or isocitric acid. Another example is the hydroxylation of nicotinic acid in Pseudomonas fluorescens [15]. In the cases mentioned as examples above the oxygen atom of the hydroxyl group is derived from water (another possibility is gaseous oxygen), and at least in the two first cases well characterized enzymes [17] are responsible for the transformation.

It can be concluded that the supernumerary carbon atoms in the methoxyl groups of anhydro-rhodovibrin, rhodovibrin, monodemethylated spirilloxanthin and spirilloxanthin are introduced by methylation of preceding hydroxy intermediates. It is of interest in this respect to note that transmethylation is a well-known and widespread biological reaction [18], contrary to transmethoxylation which apparently has not been demonstrated. Recent investigations using labelled carbon have revealed

that the methyl moiety of the methoxyl groups in the alkaloid ricinin (Ricinus communis) [19], in the lignin of tobacco leaves and barley [20] and also in mycophenolic acid (Penicillium brevi-compactum) [21] are synthesized in vivo by transmethylation. These observations are taken to indicate a rather universal occurrence of this type of reaction in living organisms. Braithwaite and Goodwin [22] have also favoured the view that the methoxyl groups of spirilloxanthin were introduced by transmethylation.

The establishment of chemical structures for the carotenoids participating in the biochemical transformation of lycopene to spirilloxanthin, unfortunately does not give any hint to the general mechanism for extension of the conjugated double bond chain. The oxidation reaction B seems to proceed rapidly, leaving no detectable intermediates behind.

A discussion of the path of carotenoid biosynthesis in Athiorhodaceae in general will be published elsewhere.

1. Liaaen Jensen, S. Acta Chem. Scand. 12 (1958) 1698.
2. Liaaen Jensen, S. Acta Chem. Scand. 13 (1959) 381.
3. Liaaen Jensen, S. Acta Chem. Scand. 13 (1959) 842.
4. Liaaen Jensen, S. Acta Chem. Scand. 13 (1959) 2142.
5. Liaaen Jensen, S. Acta Chem. Scand. 13 (1959) 2143.
6. Liaaen Jensen, S. Acta Chem. Scand. 14 (1960) 950.
7. Liaaen Jensen, S. Acta Chem. Scand. 14 (1960) 952.
8. Liaaen Jensen, S., Cohen-Bazire, G., Nakyama, T. O. M. and Stanier, R. Y. Biochim. et Biophys. Acta 29 (1958) 477.
9. Goodwin, T. W. and Land, D. G. Arch. Mikrobiol. 24 (1956) 305.
10. Zechmeister, L. and Cholnoky, L. v. Ber. 69 (1936) 422.
11. Karrer, P. and Koenig, H. Helv. Chim. Acta 23 (1940) 460.
12. Polgàr, A., van Niel, C. B. and Zechmeister, L. Arch. Biochem. 5 (1944) 243.
13. Barber, M. S., Jackman, L. M. and Weedon, B. C. L. Proc. Chem. Soc. 1959 96.
14. Liaaen Jensen, S. To be published.

15. Hunt, A. L., Hughes, D. E. and Lowenstein, J. M. *Biochem. J.* **69** (1958) 170.
16. Massart, L. and Vercauteren, R. *Ann. Rev. Biochem.* **28** (1959) 527.
17. Ochoa, S. in Summer, J. B. and Myrbäck, K. *The Enzymes.* Vol. I. Part 2, p. 217. Acad. Press, New York 1951.
18. Cantoni, G. L. *Proc. 3rd Intern. Congr. Biochem.* **1955** 233.
19. Dubeck, M. and Kirkwood, S. *J. Biol. Chem.* **199** (1952) 307.
20. Byerrum, R.U., Flokstra, J.H., Dewey, L.J. and Ball C.D. *J. Biol. Chem.* **210** (1954) 633.
21. Birch, J. A., English, R. J., Massy-Westropp, R. A., Slayton, M. and Smith, H. *J. Chem. Soc.* **1958** 365.
22. Braithwaite, G. D. and Goodwin, T. W. *Nature* **182** (1958) 1304.

Moss Anthocyanins

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From the red moss, *Bryum cryophilum* O. Mårt. (*B. obtusifolium* Lindb.) two crystalline anthocyanins have been isolated and by means of cellulose column chromatography separated from each other. One of these anthocyanins occurs in about 0.5 % of the dry weight of the moss whereas the other one occurs in a much smaller concentration, hence the latter has been investigated mainly chromatographically.

On acid hydrolysis, the two anthocyanins yield the same aglycone (identical absorption spectra, chromatographic data and colour reactions) and, according to the paper chromatogram, only one sugar, *viz.* glucose. The aglycone has been identified as luteolinidin (3′,4′,5,7-tetrahydroxyflavylium chloride) [1,2], by comparing its spectral and chromatographic data as well

as its characteristic colour reactions with those of a synthetic sample [3].

By treating the anthocyanin occurring most abundantly with hydrochloric acid for a few minutes, a mixture of pigments is obtained. When this mixture is chromatographed on paper in 1 % of hydrochloric acid it gives three spots, one of which has the same R_F-value as the unchanged pigment (0.44) and another the same as the anthocyanidin (0.03). The third spot has the same R_F-value (0.13) and the same colour as those of the other isolated anthocyanin. Furthermore, this latter gives on partial hydrolysis only two spots having R_F-values of 0.13 and 0.03, corresponding to the unchanged pigment and to the aglycone, respectively. These facts indicate that the first anthocyanin is a bioside and the second one is the corresponding monoside [4].

According to Harborne [5], anthocyanins with a free hydroxyl group in the 5-position have higher absorption intensity in the region 410−450 mμ than those having a substituted hydroxyl group in this position. Since the ratios of the optical density at 440 mμ compared to that at the wavelength of maximal absorption (E_{440}/E_{max}) for the isolated anthocyanins are only half those of the corresponding anthocyanidins, the two anthocyanins ought to be luteolinidin-5-diglucoside and luteolinidin-5-monoglucoside.

A detailed report will appear later elsewhere.

1. Pratt, D. D. and Robinson, R. *J. Chem. Soc.* **127** (1925) 1128.
2. Harborne, J. B. *John Innes Ann. Rept.* **1957** 25.
3. León, A. and Robinson, R. *J. Chem. Soc.* **133** (1931) 2732.
4. Harborne, J. B. and Sherratt, H. S. A. *Experientia* **13** (1957) 486.
5. Harborne, J. B. *Biochem. J.* **70** (1958) 22.