# Bacterial Carotenoids

## VII. A Partial Synthesis of Spirilloxanthin and OH-Spirilloxanthin

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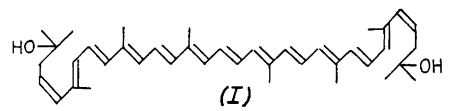
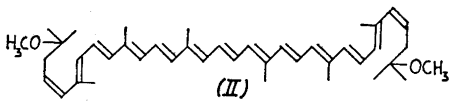
From the structure (I) ascribed to bacterioruberine *a* in the previous paper[1] it




(I)

is seen that bacterioruberine *a* represents a di-demethylated derivative of spirilloxanthin (II)[2,3].




(II)

It is obvious that a mutual support for these structures could be obtained if dimethylated bacterioruberine *a* and spirilloxanthin were shown to be identical.

Bacterioruberine *a* was isolated from a *Halobacterium* sp. (Strain No. 1, from the Department of Biochemistry, this University) as previously described[1]. Several procedures were tried to methylate this carotenoid. The instability of bacterioruberine *a* towards heat and acid catalysts restricted the number of useful methods. Treatment with diazomethane in methanol-ether[4], dimethylsulphate and potassium carbonate in dry acetone[5] or the usual method with potassium *t*-amyloxylate and methyl iodide[6] failed to give any methylation products. This was to be expected in view of the non-enolic, tertiary character of the hydroxyl groups of bacterioruberine *a*.

The careful method reported by Kuhn *et al.*[7] for the methylation of N-acetylglucosamine derivatives with methyl iodide and silver oxide in dimethylformamide proved, however, to be successful also in this case. Following this method mono- and dimethyl-derivatives of bacterioruberine *a* were obtained in maximum yields of 12 % and 2.4 %, respectively, in fourteen different methylation experiments besides unchanged starting material. The reaction was carried out at room temperature, in darkness, under nitrogen and with magnetic stirring. The methylation process was followed paper chromatographically[8] and usually interrupted after about 70 h. The carotenoids were transferred to benzene and separated by chromatography on deactivated alumina. The yields could not be significantly increased by lowering or increasing the reaction temperature, by using dry dimethylformamide or by exchanging the silver oxide for barium oxide[9], and were in any case not well reproducible.

The methylation products could only be isolated in amounts insufficient for crystallization and were characterized by means of absorption spectra in visible light, column and paper[8] chromatography of the main stereoisomers and quantitative partition tests according to Zechmeister and Petracek[10].

The properties of mono-methoxy-bacterioruberine *a* are presented in Tables 1 and 2 together with the corresponding properties of the so-called OH-spirilloxanthin, a minor pigment

*Table 1.* Absorption maxima for the main stereoisomers of mono-methoxy-bacterioruberine *a* and OH-spirilloxanthin.

| Carotenoid | Member of the stereoisomeric set | Abs.max. in mμ in acetone | | | | | Abs.max. in mμ in pet.eth.bp. 60–70°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mono-methoxy-bacterioruberine *a* | *trans* | 373 | 389 | 469 | 499 | 533 | 369 | 385 | 462 | 494 | 528 |
| | Neo A | 373 | 388 | 461 | 490 | 522 | | | | | |
| OH-spirilloxanthin | *trans* | 373 | 389 | 467 | 499 | 533 | 369 | 385 | 462 | 494 | 528 |
| | Neo A | 373 | 389 | 462 | 491 | 523 | | | | | |

*Table 2.* Properties of mono-methoxy-bacterioruberine *a* and OH-spirilloxanthin.

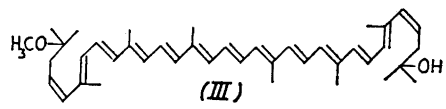
| Carotenoid | Member of the stereo-isomeric set | $R_F$-value * | | Quant. partition ratio [10] | |
|---|---|---|---|---|---|
| | | 10 % ** | 20 % ** | Pet.eth. / 95 % methanol | Pet.eth. / 85 % methanol |
| Mono-methoxy-bacterioruberine *a* | *trans* | 0.39 | 0.80 | 42:58 | 95:5 |
| | Neo A | 0.59 | 0.88 | | |
| OH-spirillo-xanthin | *trans* | 0.40 | 0.80 | 44:56 | 95:5 |
| | Neo A | 0.58 | 0.87 | | |

* on Schleicher and Schüll No. 2 87 paper.
** acetone in pet.ether b.p. 60— 70°C.

present in cells of *Rhodospirillum rubrum* [11, 12] in the exponential growth stage and in *Rhodopseudomonas palustris* [13]. These bacteria were used as sources for the isolation of chromatographically pure fractions of OH-spirilloxanthin.

The absorption spectra of the corresponding stereoisomers of mono-methoxy-bacterioruberine *a* and OH-spirilloxanthin further had analogous shapes. Co-chromatography of the *trans* isomers of the two pigments gave one zone which could not be resolved on kiselguhr paper. The solvents required for elution from deactivated alumina also were similar. The partition tests indicate the presence of one hydroxyl group in both pigments. Both pigments gave the same products upon HCl—$CHCl_3$ treatment [14].

The data presented for mono-methoxy-bacterioruberine *a* and OH-spirilloxanthin strongly suggest that these pigments are identical, leading to a structural formula (III) for OH-spirilloxanthin



(III)

as a mono-demethylated spirilloxanthin — in good agreement with the previous tentative assumption [11] and the kinetic data for its biosynthesis in *Rhodospirillum rubrum* [12] from rhodovibrin (OH—P481) [15] and as a spirilloxanthin precursor.

*Table 3.* Absorption maxima for the main stereoisomers of di-methoxy-bacterioruberine *a* and spirilloxanthin.

| Carotenoid | Member of the stereo-isomeric set | Abs.max. in mμ in acetone | | | | | Abs.max. in mμ in pet.eth. b.p. 60—70°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Di-methoxy-bacterio-ruberine *a* | *trans* | 373 | 389 | 468 | 498 | 533 | 369 | 385 | 462 | 494 | 527 |
| | Neo A | 372 | 389 | 468 | 496 | 529 | | | | | |
| | Neo B | 372 | 389 | 464 | 489 | 523 | | | | | |
| Spirillo-xanthin | *trans* | 373 | 389 | 468 | 498 | 533 | 369 | 385 | 462 | 494 | 528 |
| | Neo a[8] | 372 | 389 | 467 | 495 | 529 | | | | | |
| | Neo b[8] | 372 | 389 | 462 | 489 | 523 | | | | | |

*Table 4.* Properties of di-methoxy-bacterioruberine *a* and spirilloxanthin.

| Carotenoid | Member of the stereo-isomeric set | $R_F$-value * 5 % * | $R_F$-value * 10 % ** | Quant.partition ratio [10] Pet.eth./95 % methanol |
|---|---|---|---|---|
| Di-methoxy-bacterio-ruberine *a* | *trans* | 0.40 | 0.76 | 84:16 |
| | Neo A | 0.54 | | |
| | Neo B | 0.71 | | |
| Spirillo-xanthin | *trans* | 0.40 | 0.76 | 88:14 |
| | Neo a[8] | 0.54 | | |
| | Neo b[8] | 0.73 | | |

* on Schleicher and Schüll No. 287 paper.
** acetone in pet.ether b.p. 60—70°C.

Similar properties for di-methoxy-bacterio-ruberine *a* and spirilloxanthin are presented in Tables 3 and 4. Spirilloxanthin was isolated from the same source as OH-spirillo-xanthin as previously described[8]. The shape of the absorption spectra of the corresponding stereoisomers of the two carotenoids were very similar.

Co-chromatography of the *trans* isomers on kiselguhr paper gave one zone which could not be resolved. Di-methoxy-bacterioruberine *a* and spirilloxanthin further required similar solvents for elution from deactivated alumina.

The data presented are greatly in favour of identity of di-methoxy-bacterioruberine *a* and spirilloxanthin, a fact that strengthens the suggested structures for bacterio-ruberine *a* (I) and spirilloxanthin (II).

This work will be published in more detail elsewhere.

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1. Liaaen Jensen, S. *Acta Chem. Scand.* **14** (1960) 950.
2. Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 381.
3. Barber, M. S., Jackman, L. M. and Weedon, B. C. L. *Proc. Chem. Soc.* **1959** 96.
4. Eistert, B. *Neuere Meth. der Präp. Org. Chem.* **1**, Verlag Chemie, Berlin 1943, p. 364.
5. *cf.* Seshadri, T. R. and Subramanian, S. S. *Proc. Indian Acad. Sci.* **30A** (1949) 67.
6. Karrer, P. and Takahashi, T. *Helv. Chim. Acta* **16** (1933) 1163.
7. Kuhn, R., Trischmann, H. and Löw, I. T. *Angew. Chem.* **67** (1955) 32.
8. Jensen, A. and Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 1863.
9. Kuhn, R., Baer, H. H. and Seeliger, A. *Ann.* **611** (1958) 236.
10. Zechmeister, L. and Petracek, F. J. *Anal. Chem.* **28** (1956) 1484.
11. van Niel, C. B., Goodwin, T. W. and Sissins, M. E. *Biochem. J.* **63** (1956) 408.
12. Liaaen Jensen, S., Cohen-Bazire, G., Nakayama, T. O. M. and Stanier, R. Y. *Biochem. et Biophys. Acta* **29** (1958) 477.
13. Goodwin, T. W. *Soc. Biol. Chemists, India. Silver Jubilee Souvenir* **1955** 698.
14. Wallcave, L. and Zechmeister, L. *J. Am. Chem. Soc.* **75** (1953) 4495.
15. Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 2143.

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