Bacterial Carotenoids

VI. A Note on the Constitution of Bacterioruberine *α*

SYNNÖVE LIAAEN JENSEN

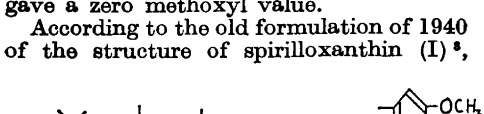
Institutt for Organisk Kjemi, Norges Tekniske Högskole, Trondheim, Norway

This paper gives a preliminary report on the chemical constitution of bacterioruberine *α* — the main carotenoid pigment of certain red, rod-shaped, obligate and extremely halophilic bacteria for which Volcani [1] have suggested the genus name *Halobacterium*.

In 1932 Petter [2] isolated two crystalline carotenoids named bacterioruberine *α* (abs. max. 460, 490, 528 mμ in methanol) and bacterioruberine *β* (abs.max. 452, 482, 522 mμ in methanol) from a bacterium which she named *Bacterium halobium*, and which undoubtedly belongs to the genus *Halobacterium* (Volcani). From the same source Lederer [3] could isolate only crystalline bacterioruberine *α* (abs.max. 460, 495, 528 mμ in methanol). The absorption spectrum was found to be similar to that of rhodoviolascin [4], later shown to be identical with spirilloxanthin [5]. Bacterioruberine *α*, however, had a pronounced hypophasic character, and Lederer suggested that this carotenoid possibly was a di-demethylated spirilloxanthin.

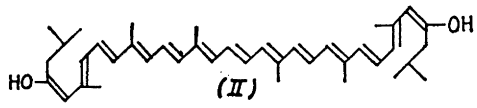
A carotenoid with absorption spectrum similar to that of bacterioruberine *α* was present also in cells of an obligate, halophilic bacterium studied by Spruit and Pijper [6]. An interesting chromatographic survey of the carotenoids of a number of halophilic bacteria has recently been given by Baxter [7], again stating that bacterioruberine *α* is the main carotenoid of several red *Halobacterium* species. A sample of bacterioruberine *α* of unreported purity gave a zero methoxyl value.

According to the old formulation of 1940 of the structure of spirilloxanthin (I) [8],



(I)

Lederer's suggestion of 1938 that bacterioruberine *α* was a di-demethylated spirilloxanthin would imply the structure (II) for this carotenoid with two enolic



(II)

hydroxyl groups. This has not been confirmed by the present investigation.

In the present investigation bacterioruberine *α* was isolated as the major carotenoid from a *Halobacterium* sp. (Strain No. 1, from the Department of Biochemistry, this University). The carotenoid was extracted with acetone and purified by chromatography of the unsaponifiable matter on deactivated alumina.

Bacterioruberine *α* crystallised with difficulty as the all-*trans* isomer from acetone-petroleum ether solution in mauve-violet, shiny, needles, m. p. 182°C (evacuated tube). Consistent micro combustion analyses could not be obtained. The crystalline carotenoid was readily soluble in pyridine and acetone, fairly readily soluble in methanol, chloroform and carbon disulphide, fairly soluble in benzene and diethyl ether and nearly insoluble in petroleum ether. Absorption maxima in various solvents as recorded immediately after dissolution with a Zeiss PMQ2 calibrated spectrophotometer is presented in Table 1 together with similar data reported for spirilloxanthin [5]. The agreement in absorption maxima for the two carotenoids falls within the experimental errors for different samples and instruments. The shape of the absorption spectra in the utilised solvents was further identical and showed pronounced fine-structure. It appears therefore that bacterioruberine *α* and spirilloxanthin have identical chromophores, that is 13 conjugated carbon-carbon double bonds in an aliphatic chain.

Quantitative extinction coefficient determinations carried out in acetone gave as a maximum value $E^{1\%}_{1\,cm} = 2\,620$ at 499 mμ, a value within the expected range for a di-demethylated spirilloxanthin. Spirilloxanthin in hexane has $E^{1\%}_{1\,cm} = 2\,540$ at 493 mμ [5].

A stereochemical investigation of bacterioruberine *α* was performed by means of a paper-chromatographical method previously reported [9]. Bacterioruberine *α* was found to exhibit a greater stereochemical lability than any other carotenoid so far reported. It might be mentioned that after 24 h in darkness at room temperature the all-*trans* isomer had isomerized spontaneously to Neo U to an extent of 42 % as colorimetrically determined after ra-

Table 1. Absorption maxima in various solvents for all-*trans* bacterioruberine *a* and spirilloxanthin [5].

Carotenoid	Abs.max. in mμ in				
	Pet.ether *	Benzene	Acetone	$CHCl_3$	CS_2
Bacterioruberine *a*	369	378	374	380	
	385	398	389	397	418
	461	481	466	475	500.5
	494	511	499	506	533.5
	528	549	533.5	544	572
Spirilloxanthin	368	378			
	384	395			(418)
	461	479		(475)	495
	493	510		505	532
	528	548.5		543	571.5

* b.p. 60—70°C.

pid chromatographic separation. After 24 h exposure to indirect daylight at room temperature of a solution of the pure *trans* isomer, only 37 % *trans* remained, whereas gross stereoisomerisation to Neo U, Neo A and Neo B had occurred. The composition of the iodine catalyzed equilibrium mixture is presented in Table 2.

The two first maxima give the position of the *cis*-peak, which is very weak for the *trans* isomer and strongest for the Neo U isomer. R_F-values for the different stereoisomers have been published previously [9].

Quantitative partition tests according to the method of Zechmeister and Petracek [10] gave the following partition ratios:

Pet.ether/95 % methanol	1:99
Pet.ether/85 % methanol	3:99

Bacterioruberine *a* is seen to be strongly hypophasic even with 85 % methanol, thus indicating the presence of two or more hydroxy groups in the molecule.

Upon prolonged treatment with acetic anhydride in pyridine bacterioruberine *a* was completely recovered; whereas bacterioruberine *a* was very unstable towards freshly distilled acetyl chloride in pyridine, yielding only decomposition products. The failure of bacterioruberine *a* to yield an acetate indicated that all hydroxyl groups present were tertiary. This was confirmed by the IR-spectrum (KBr) which showed a strong absorption band for the hydroxyl stretching frequency at 3 320 cm^{-1}, no absorption band around 1 030 cm^{-1} characteristic of secondary hydroxyl groups [11], but a fairly strong band typical of tertiary hydroxyl groups at 1 142 cm^{-1} as in chloroxanthin [11], rhodopin [11] and rhodovibrin [12].

$HCl—CHCl_3$-treatment [13] gave no product with extended conjugated chain. Allylic hydroxyl groups are therefore not likely to be present.

Table 2. Composition of the iodine catalyzed equilibrium mixture of bacteriorubine *a*.

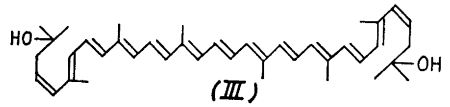
Member of the stereoisomeric set	Abs.max. in mμ in		% of total colorimetr. determined *
	pet.eth. bp. 60—70°C	acetone	
Neo B	369, 385, 463, 492, 522	374, 389, 470, 498, 526	1
Neo A	369, 385, 454, 483, 516	374, 389, 460, 489, 522	20
all-*trans*	369, 385, 461, 494, 528	374, 389, 466, 499, 533.5	37
Neo U	369, 385, 460, 490, 522	374, 389, 466, 496, 527	42

* The same extinction coefficient was used for calculating the actual amount of each stereoisomer.

The stability of the chromophore of bacterioruberine *a* in neutral as well as in basic media further refuted the presence of enolic hydroxyl groups [14]. The absence of enolic hydroxyl groups was supported also by the lack of a relatively intense IR-absorption band around 1 660 cm^{-1} attributed by Rosenkrantz and Gut [15] to the grouping $-CH=C-O-$.

The IR-spectrum further showed that no methoxyl groups or carbonyl functions were present in bacterioruberine *a*.

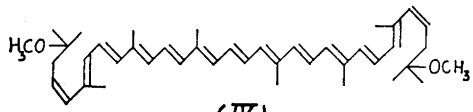
Combining the evidence of a chromophore of 13 conjugated carbon-carbon double bonds in an aliphatic system with the presence of at least two non-allylic, non-enolic, tertiary hydroxyl groups, it seems that only one structure (III) is probable for bacterioruberine *a*.



(III)

The presence of no more than two hydroxyl groups was further confirmed by quantitative determination of active hydrogen. (Found: Active H 0.39 %. Calc. for $C_{40}H_{54}(OH)_2$: 0.35 %; for $C_{40}H_{52}(OH)_3$: 0.51 %.)

From the new spirilloxanthin structure (IV) [14, 16] it is thus seen that bacterioruberine *a* should be a di-demethylated spirilloxanthin in agreement with Lederer's [3] original suggestion.



(IV)

Bacterioruberine *a* is another example of a bacterial carotenoid with tertiary hydroxyl groups in addition to chloroxanthin [17, 11], rhodopin [11], rhodovibrin [12] and OH-spirilloxanthin [18] from photosynthetic bacteria. This appears to be a characteristic feature so far not reported for carotenoids from higher organisms. The position of the hydroxyl group in chloroxanthin has not yet been established, but is for the rest of the carotenoids mentioned above invariably located in 1-positions.

This work will be published in more detail elsewhere.

The author is deeply indebted to Siv.ing. Ian Dundas at the Department of Biochemistry for the cultivation of the *Halobacterium* sp., to Professor N. A. Sörensen for his inspiring interest in this work and to Norges Tekniske Högskole for a maintenance grant.

1. Elazari-Volcani, B. *Studies on the Microflora of the Dead Sea, Thesis*, Hebrew University, Jerusalem 1940.
2. Petter, H. F. M. *Over roode en andere bacteriën van gezouten vish, Thesis*, Utrecht 1932.
3. Lederer, E. *Bull. soc. chim. biol.* **20** (1938) 611.
4. Karrer, P. and Solmssen, U. *Helv. Chim. Acta* **19** (1935) 219.
5. Polgàr, A., van Niel, C. B. and Zechmeister, L. *Arch. Biochem.* **5** (1944) 243.
6. Spruit, C. J. P. and Pijper, A. *Antonie van Leeuwenhoek* **18** (1952) 190.
7. Baxter, R. M. *Can. J. Microbiol. In press.*
8. Karrer, P. and Koenig, H. *Helv. Chim. Acta* **23** (1940) 460.
9. Jensen, A. and Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 1863.
10. Zechmeister, L. and Petracek, F. J. *Anal. Chem.* **28** (1956) 1484.
11. Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 842.
12. Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 2143.
13. Wallcave, L. and Zechmeister, L. *J. Am. Chem. Soc.* **75** (1953) 4495.
14. Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 381.
15. Rosenkrantz, H. and Gut, M. *Helv. Chim. Acta* **36** (1953) 1000.
16. Barber, M. S., Jackman, L. M. and Weedon, B. C. L. *Proc. Chem. Soc.* **1959** 96.
17. Nakayama, T. O. M. *Arch. Biochem. Biophys.* **75** (1958) 352.
18. Liaaen Jensen, S. *Acta Chem. Scand.* **14** (1960) 953.

Received May 5, 1960.