

## Bacterial Carotenoids

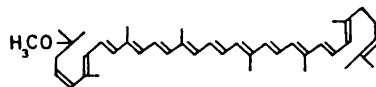
## IX. The Constitution of the Third Member of the P481-Group (3,4-Dehydro-Rhodopin)

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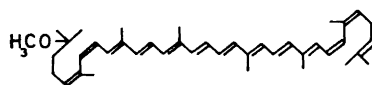
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Goodwin<sup>1,2</sup> has shown that several species of the photosynthetic purple bacteria contain two carotenoids previously referred to as P481 and OH-P481. Work on the chemical constitution of these carotenoids has led to the suggestions of structures (I or II) for P481<sup>3,4,12</sup> and (III or IV) for OH-P481<sup>5</sup>. The latter carotenoid is assumed to be identical with rhodovibrin described by Karrer, Solmssen and Koenig<sup>6-8,9,5</sup>. On the basis of the structural

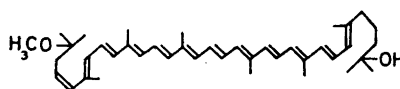
relationship between rhodovibrin (III or IV) and P481 (I or II) the name anhydro-rhodovibrin has been suggested for P481<sup>4,10</sup>.



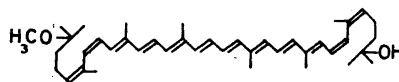
(I)



(II)



(III)



(IV)

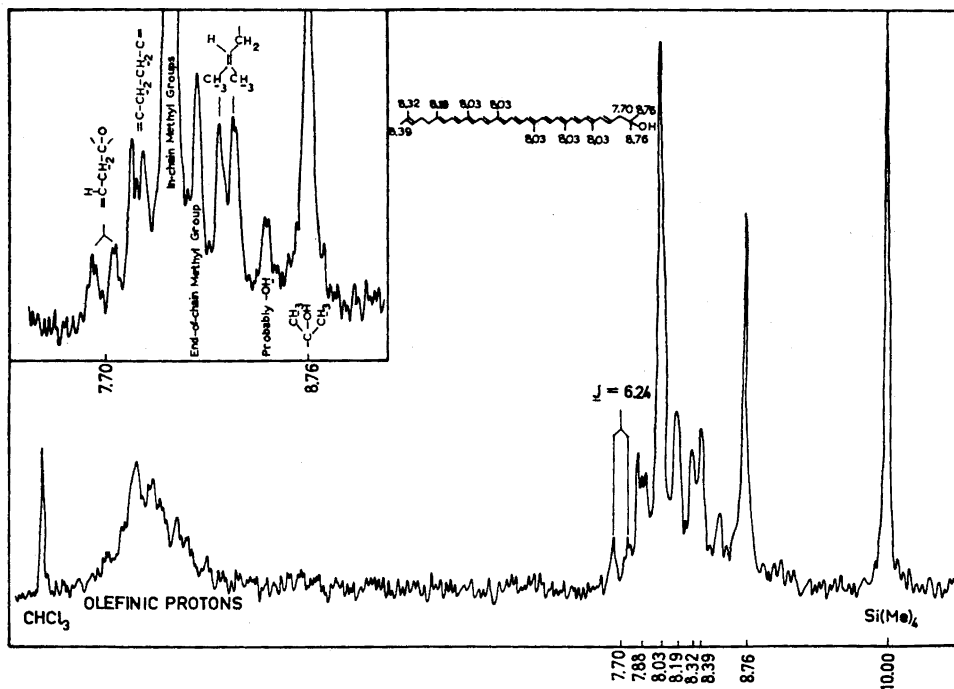
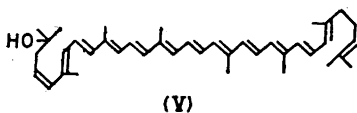


Fig. 1. The proton spectrum of 3,4-dehydro-rhodopin. 4.1 mg/0.19 ml  $\text{CDCl}_3$  at 60 Mc/s.

For biochemical reasons, the structures (I) and (III), with unsymmetrically located chromophores, for anhydro-rhodovibrin and rhodovibrin respectively, have been preferred<sup>10,11</sup>, although the chemical evidence does not support such a preference. It was hoped that conclusive evidence for the position of the chromophore could be obtained from the NMR-spectrum of rhodovibrin. Such a study was therefore performed on a specimen isolated from a strain of *Rhodospseudomonas palustris*. This strain had been isolated in pure culture by C. B. van Niel from an impure culture provided by H. Gaffron, and which Gaffron had designated as *Rhodovibrio parvus*. The isolation was carried out as described elsewhere<sup>5,11</sup>. The yield obtained was 11.5 mg, m.p. 186–190°C (evacuated tube),  $\lambda_{\max}$  (358) (374) 455, 483 and 517 m $\mu$  in petroleum ether, with pronounced fine-structure,  $E_{1\text{ cm}}^{1\%} = 3\ 100$  at 483 m $\mu$ .

The NMR-spectrum at 60 Mc/s, recorded in CDCl<sub>3</sub> from which traces of acid had been removed, is presented in Fig. 1. The doublet ( $J = 6.2$  c/s.) at 7.70\*, and the sharp singlet at 8.76 are characteristic of the end groups of spirilloxanthin<sup>12,14</sup>, while the absorptions at 7.88, 8.19, 8.32 and 8.39 correspond closely to the end group absorptions of lycopene<sup>13,15</sup>. That the spectrum exhibits no absorption between 5.0 and 7.0 proves the absence of a methoxyl group. These facts, together with the visible light absorption data, lead unambiguously to (V) as the structure of the compound investigated.



The question immediately arises as to whether this carotenoid is identical with rhodovibrin, for which structures (III or IV) had been suggested<sup>5</sup>. The isopropylidene value and hydrogenation data obtained for rhodovibrin<sup>5</sup> are not in favour of this identity. Furthermore, the infrared absorption band at 1 078 cm<sup>-1</sup> (KBr), characteristic of tertiary methoxyl groups<sup>5,11</sup>, and the methoxyl value obtained<sup>5</sup>, would require the capture of one molecule of methanol of crystallization during

the saponification procedure. To check the latter possibility, lutein, crystallized from ether-methanol, was prepared. When crystallized from this solvent lutein is known to retain one molecule of methanol per carotenoid molecule<sup>16</sup>. The infrared spectrum of such lutein exhibited a weak band at 1 120 cm<sup>-1</sup> when measured in CHCl<sub>3</sub>. This difference in the methoxyl region of the infrared spectra of lutein·CH<sub>3</sub>OH and of rhodovibrin thus supports the contention that rhodovibrin possesses a methoxyl group.

A clear demonstration of the non-identity of (V) and rhodovibrin (III or IV) was obtained by mixed chromatography on Schleicher and Schüll No. 287 paper<sup>17</sup>. *Trans* rhodovibrin and *trans* (V) showed  $R_F$ -values 0.54 and 0.70, respectively, when the chromatogram was developed with 10 % acetone in petroleum ether. That this discrepancy in  $R_F$ -values was not detected before was due the similarity in other properties, together with the fact that, at the time when compound (V) was isolated, a new batch of paper with weaker adsorptive properties was used.

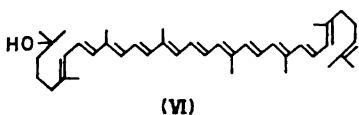
The elementary analysis reported for rhodovibrin<sup>5</sup> (C 85.88; H 10.16; O indirect 3.96; direct 3.61) was obtained on substance (V). It is seen that the result fits better with the calculated values for (V) (C<sub>40</sub>H<sub>56</sub>O: C 86.90; H 10.21; O 2.89). The calculated values for rhodovibrin (C<sub>41</sub>H<sub>56</sub>O<sub>2</sub>) are C 84.20; H 10.34; O 5.47. Also the extinction coefficient reported for rhodovibrin<sup>5</sup> ( $E_{1\text{ cm}}^{1\%} = 3\ 100$  at 483 m $\mu$  in petroleum ether) was measured on substance (V). For rhodovibrin itself the corresponding highest extinction value observed was  $E_{1\text{ cm}}^{1\%} = 2\ 910$ . Thus the molar extinction coefficients at the main maximum for compound (V) ( $\epsilon = 17\ 110$ ) and rhodovibrin ( $\epsilon = 17\ 000$ ) are in good agreement.

Compound (V) was isolated from the above mentioned strain of *Rhodospseudomonas palustris*, whereas rhodovibrin (III or IV) was isolated from *Rhodospirillum rubrum* strain 1, 1, 1, and also from *Rhodospseudomonas palustris* strain 5 (all strains of Prof. C. B. van Niels collection). An inspection of the biosynthetic scheme suggested for the transformation of lycopene to spirilloxanthin in photosynthetic purple bacteria<sup>4,10,11</sup>, reveals that compound (V) represents the hypothetical intermediate, not isolated before. Thus there is good reason to believe that compound (V)

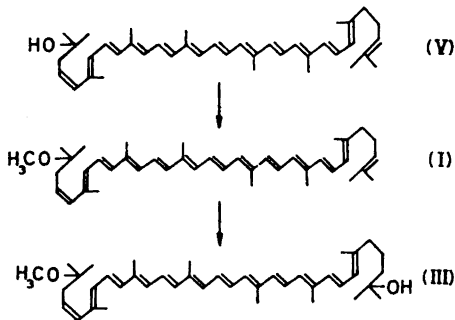
\* Line positions are quoted as  $\tau$ -values.

can be found at suitable stages of development in all the organisms where these transformations take place. The adsorptive and solubility properties of rhodovibrin (III or IV) and of (V) are probably such that under similar conditions the isolation of rhodovibrin is more readily obtained.

The compound described in this paper is the third member of the P481-group of carotenoids, and structurally is a 1,2-dihydro-3,4-dehydro-1-hydroxy-lycopene. On the basis of structure (VI) ascribed to rhodopin<sup>18,19</sup>, it can also be considered as a 3,4-dehydro-rhodopin.



The existence of (V) strengthens the biochemical arguments<sup>10,11</sup> in support of the unsymmetrical location of the chromophores in anhydro-rhodovibrin and rhodovibrin. The known members of the P481-group of bacterial carotenoids can then be represented as follows:



The biosynthetic relationship between these three members is discussed elsewhere<sup>4,10,11</sup>.

The authors wish to stress that the isolation of 3,4-dehydro-rhodopin is wholly due to the success of Prof. C. B. van Niel, Hopkins

Marine Station, Pacific Grove, California, in isolating the strain used in pure culture and to his generosity in providing dried cells for pigment extraction. One of us (SLJ) is grateful to *Norges Teknisk-Naturvitenskapelige Forskningsråd* for a maintenance grant.

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