An additional support of the non-identity of the two carotenoids was obtained by a paper chromatographical study [7] of the stereoisomers obtained by iodine catalysis of the all-*trans* compounds. The composition of the iodine catalyzed equilibrium mixtures was different for the two compounds both qualitatively and quantitatively.

As a consequence of the above study it may be considered as proved that lycoxanthin and rhodopin are not identical. This further strenghtens the suggested structure (II) for rhodopin.

This work will be published in more detail elsewhere.

The author wishes to express her gratitude to Prof. N. A. Sörensen for collecting the berries of *Solanum dulcamara* and for his inspiring interest in this work. Dried cells of two strains of *Rhodopseudomonas palustris* most kindly were supplied by Dr. C. B. van Niel, Hopkins Marine Station, Pacific Grove, California, USA. A maintenance grant from *Norges Tekniske Högskole* is gratefully acknowledged.

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Bacterial Carotenoids

V. A Note on the Constitution of Rhodovibrin (OH-P481)

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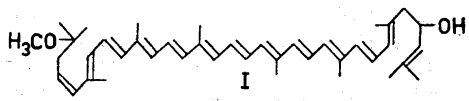
During their investigations on the carotenoids of the purple bacteria, Karrer *et al.*[1-3] described a polyene alcohol with absorption maxima in carbon disulphide at 517 and 556 mμ. In the chromatographic purification procedure employed by these workers, this carotenoid could be separated only with great difficulty from the upper part of the rhodopin zone. A crystalline sample, by Karrer *et al.* considered not to be completely pure, melted at 168°C. Combustion analysis gave 83.97 % C and 10.09 % H, which suggested that two oxygen atoms were present in the carotenoid molecule. According to partition tests, not more than one hydroxyl group could be present. Methoxyl determination was negative. This carotenoid which was rather well characterized in the early work of Karrer *et al.*, was named rhodovibrin.

From a number of purple bacteria, Goodwin *et al.*[4,5] later isolated a similar mono-hydroxy-carotenoid, designated OH-P481. Goodwin and Land [5] suggested that OH-P481 might be identical with rhodovibrin.

OH-P481 was shown by Stanier and collaborators [6] to be an intermediate between lycopene and spirilloxanthin in the biosynthesis of carotenoids in *Rhodospirillum rubrum*. It was pointed out that the absorption spectrum of OH-P481 in visible light corresponded to a chromophore of twelve conjugated carbon-carbon double bonds in an aliphatic system. The presence of one methoxyl group in OH-P481 has been established later [7].

In a speculative transformation scheme for the biochemical conversion of lycopene to spirilloxanthin, Weedon and collaborators [8] on basis of the properties previously reported for OH-P481 [5-7], suggested the structure (I) for this carotenoid.



I

This structure, which contains a secondary hydroxyl group and one *iso*propropylidene end-group is, however, not in agreement with the chemical evidence presented here.

In the present investigation OH-P481 has been isolated from cells of *Rhodospirillum rubrum* in the exponential growth stage and from dried cells of two strains of *Rhodopseudomonas palustris*. The carotenoid was extracted with acetone and isolated from the unsaponifiable matter by repeated chromatography on deactivated alumina. It crystallized as dark,

red needles from petroleum ether-acetone solution. The chromatographic fractions of OH-P481 were accompanied by oily substances in the *Rhodospirillum rubrum* extracts and a white substance mp. 282°C (subl.) in the *Rhodopseudomonas palustris* extracts, which reduced the yield of the pure, crystalline pigment. After several recrystallizations the carotenoid melted at 190.5°C.

Crystalline OH-P481 was readily soluble in acetone and carbon disulphide, fairly soluble in chloroform, benzene and methanol, less soluble in ether and nearly insoluble in cold petroleum ether.

Absorption maxima for OH-P481 in various solvents as recorded quickly after dissolution is given in Table 1. The spectra were determined in a Zeiss PMQ2 spectrophotometer. Reading of correct maxima was checked by a didymium standard filter.

Table 1. The absorption maxima of all-*trans* OH-P481 in various solvents.

Solvent	Absorption maxima in mμ	
Petroleum ether bp. 60–70°C	358 374	455 483 516
Acetone	363 378	460 488 522
Chloroform	370 385	469 498 532
Benzene	372 388	473 503 535
Carbon disulphide	(408)	491 522 559

The two maxima in the first column indicate the position of the *cis*-peak, which was very weak in the spectrum of the all-*trans* compound. The purest sample measured had $E^{1\%}_{1\ cm} = 2\,940$ at 483 mμ in petroleum ether. This sample contained 5.06 % ash as determined by combustion. When corrected for 5.06 % ash the value $E^{1\%}_{1\ cm} = 3\,100$ is obtained. By extrapolation between the extinction coefficients for spirilloxanthin $E^{1\%}_{1\ cm} = 2\,620$ [9] and lycopene $E^{1\%}_{1\ cm} = 3\,460$ [10] at λ_{max} in petroleum ether, and correcting for the presence of an additional oxygen atom the value $E^{1\%}_{1\ cm} = 2\,970$ is obtained. It is therefore likely that the value $E^{1\ cm}_{1\%} = 3\,100$ for OH-P481 at λ_{max} in petroleum ether is close to the correct value. Iodine catalysis caused a shift to 358, 374, (455), 479 and 511 mμ in petroleum ether with 25 % decrease in extinction coefficient at the middle maximum. OH-P481 crystallizes normally in the all-*trans* form, but was readily *cis*-isomerized in solution. The stereochemical lability was studied by means of the paper-chromatographical method reported by Jensen *et al.*[11] The main *cis*-isomers designated Neo A and Neo B showed absorption maxima in acetone at 376, 459, 485, 517 mμ and 376, 453, 481, 513 mμ, respectivly. In the iodine catalyzed equilibrium mixture a content of 58 % all-*trans*, 18 % Neo A and 24 % Neo B was determined colourimetrically. Otherwise the stereochemical lability was comparable with that of spirilloxanthin [9].

In the quantitative partition test recommended by Zechmeister and Petracek [12], the following partition ratio was found:

Petroleum ether/95 % methanol	66:34
Petroleum ether/85 % methanol	95:5

The carotenoid thus distributed as a monohydroxy-carotenoid. The presence of a hydroxyl group further was established by an IR absorption band at 3 420 cm^{-1} in KBr. There was, however, no absorption band in the 1 030 cm^{-1} region, which is characteristic for secondary hydroxyl groups, but a band of weaker intensity at 1 148 cm^{-1}, which previously has been discussed and found typical of carotenoids containing tertiary OH-groups [13]. The tertiary character of the hydroxyl group further was confirmed by the behaviour of the pigment upon acetylation. A series of attempts to acetylate OH-P481 with acetic anhydride or acetyl chloride in pyridine, never gave a compound with properties corresponding to an acetate in yields exceeding 5 % of the unchanged starting material. This resistance to acetylation is similar to that reported for rhodopin [3,13,14] with a tertiary hydroxyl group. Parallel experiments with lutein resulted in quantitative formation of lutein diacetate.

Treatment with $CHCl_3$-HCl according to Entschel and Karrer [15] gave no product with extended conjugated chain. Neither the hydroxyl group, nor the methoxyl group of OH-P481 are therefore likely to be allylic.

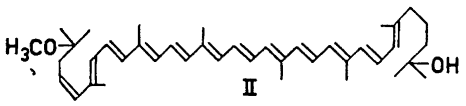
Quantitative determination of *iso*propylidene groups according to the method of Kuhn and Roth [16] gave 0.62 moles of acetone per mole of carotenoid compared with the value 1.70 simultaneously obtained for lycopene which contains two *iso*propylidene end-groups. The value obtained for OH-P481 is lower than expected for one *iso*propylidene group, and is interpreted as being due to the presence of one end-group with a tertiary methoxyl group as in spirilloxanthin [6,17] and another containing a tertiary hydroxyl group as in rhodopin [13].

The possibility for these types of end-groups to yield a small amount of acetone upon ozonolysis has already been pointed out.[17,18]

Catalytic micro-hydrogenation of a specimen with $E_{1\ cm}^{1\%} = 2\,850$ at 483 mμ in petroleum ether gave an uptake of 11.32 and 11.40 moles of hydrogen per mole of carotenoid calculated on the basis of a molecular formula $C_{40}H_{56}(OH)(OCH_3)$. The absorption spectrum in visible light indicates a chromophoric system containing twelve conjugated double bonds, hence the hydrogenation value is obviously somewhat low. Accepting $E_{1\ cm}^{1\%} = 3\,100$ for the pure pigment, the sample used in the hydrogenation has been 92 % pure. Chromatographic purity tests had revealed that the impurities present in the sample were not of carotenoid nature. The hydrogen uptake could then be corrected to a maximum of 12.3 moles per mole of carotenoid, assuming that the impurities do not consume any hydrogen.

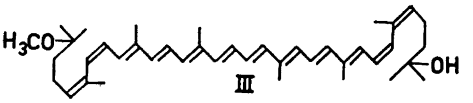
Exact analytical data could not be obtained because of the difficulty of isolating an ash-free substance. The figures given below are corrected for the presence of 5.06 % ash of unknown composition. (Found: C 85.88, H 10.16, O indirect 3.96, direct 3.61. Calc. for $C_{40}H_{56}(OH)(OCH_3)$: C 84.20, H 10.34, O 5.47.) The values obtained correspond to a molecular formula $C_{41}H_{60}O_{1.4}$. The presence of two oxygen atoms in the molecule is, however, clearly demonstrated from (1) the quantitative determination of methoxyl [7], and (2) partition behaviour, chromatographic behaviour, IR-spectrum and acetylation which reveal the presence of a hydroxyl group.

As a consequence of the data presented above, the structure (II) is possible for OH-P481:



II

This formulation is consistent with a chromophoric system of twelve conjugated double bonds in an aliphatic system, one non-allylic methoxyl group at a similar position as in spirilloxanthin, one non-allylic, tertiary hydroxyl group and no *iso*propylidene end-group. The available chemical data, however, do not exclude a symmetrical position of the chromophore as in (III).



III

The structures (II) or (III) for OH-P481 together with the structures previously suggested for rhodopin [13], P481 [8,17] and spirilloxanthin [8,17] make possible a more definite interpretation in chemical terms of the reactions involved in the later steps of carotenoid biosynthesis in the genus *Rhodospirillum* [16], for which the kinetics already has been established [6].

In view of the similar source of isolation, the accompanying carotenoids, the visible absorption spectrum, partition tests and chromatographic behaviour, there is good reason to believe that OH-P481 is identical with rhodovibrin, first described by Karrer and Solmssen [1]. As long as rhodovibrin was not isolated from a definite organism and not in a completely pure state, this cannot be definitely proved. In accordance with the generally accepted nomenclature it is suggested that OH-P481 from now on should be referred to as rhodovibrin. A further reason for changing the name of OH-P481 is the fact that the main absorption maximum of the all-*trans* compound is located at 483 mμ and not at 481 mμ in petroleum ether.

This work will be published in more detail elsewhere.

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Note on the Crystal Structure of Vanadium Dichloride

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In a paper on transition metal halides [1] Klemm and Grimm were unable to determine the structure of VCl_2 and $TiCl_2$. They stated, however, that VCl_2 was not isomorphous with TiI_2 which was found to have the CdI_2-type structure. Later Baenziger and Rundle have found a preparation of $TiCl_2$ to be isomorphous with CdI_2[2].

We have carried out an X-ray investigation of VCl_2 prepared by K. Riishede of this laboratory by heating vanadium powder in a stream of dry HCl at 950°C. The product was obtained in the form of apple-green, muscovite-like leaflets of a more or less hexagonal appearance. The following data were obtained:

Unit cell dimensions: $a_0 = 3.60 \pm 0.01$ Å, $c_0 = 5.83 \pm 0.01$ Å. Observed density[1]: 3.09 g/cm³, calculated density: 3.09 g/cm³. Diffraction symbol: $\bar{3}mP...$; test for pyroelectricity: negative. Probable space group: $P\bar{3}m$ (No. 164). Arrangement of atoms:

1 V at 0,0,0
2 Cl at $\pm$ (1/3, 2/3, u) with $u \sim 1/4$.

Shortest V-Cl distance: 2.55 ± 0.05 Å. Structure type: CdI_2 ($C6$).

Experimental: Laue photographs were taken along the c-axis. The lattice constants were calculated from Debye-Scherrer diagrams taken in a 19 cm camera using Cr$K\alpha$ radiation ($\lambda = 2.2909$ Å). Intensities were recorded on an X-ray diffractometer with Cu$K\alpha$ radiation. The value of the parameter u was found to deviate only insignificantly from the ideal value 1/4 from a Fourier projection along the c-axis using the $00l$ reflections. The agreement between observed and calculated intensities of the $00l$ and $10l$ reflections is satisfactory but a different scaling factor had to be used because preferred orientation could not be totally depressed.

From the above it may be concluded either that $TiCl_2$ and VCl_2 both exist in two modifications or that the materials investigated by Klemm and Grimm have been partly decomposed before or during their X-ray exposure.

Added in proof: Very recently[3] Ehrlich and Seifert have recorded the structure of VCl_2 in complete agreement with the results given above.

The author is indebted to the head of the Chemistry Department of the Royal Veterinary and Agricultural College in Copenhagen, professor A. Tovborg Jensen, for permission to use the X-ray diffractometer there and to Mr. Haldor Topsøe for permission to publish this note.

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