Bacterial Carotenoids

IV *. A Comparative Study of Lycoxanthin and Rhodopin

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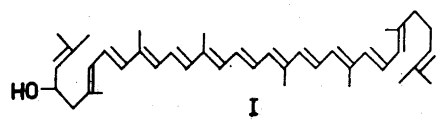
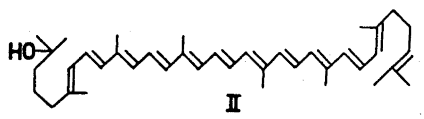
It has been claimed by Goodwin and Land [1] that lycoxanthin (I), first isolated by Zechmeister and Cholnoky [2] from *Solanum dulcamara*, and rhodopin, first isolated by Karrer and Solmssen [3] from purple bacteria, were identical.



I

A critical examination of the literature [1-6] reveals, however, certain data contradictory to this statement. This has recently been pointed out by the present author [6], who proposed the structure (II) for rhodopin in accordance with the properties reported for this carotenoid [1,3-6].



II

It was therefore found desirable to repeat a direct, comparative study of the two carotenoids.

In the present investigation lycoxanthin has been isolated from *Solanum dulcamara*, and rhodopin from *Rhodospirillum rubrum* in the exponential growth stage and also from dried cells of two strains of *Rhodopseudomonas palustris*, as previously described [6].

Lycoxanthin was isolated from the ripe berries of *Solanum dulcamara* by acetone extraction followed by chromatography of the unsaponifiable matter on deactivated alumina. Lycoxanthin crystallized in dark, red needles mp. 167.5—168.5 °C with λ_{max} in petroleum ether at (422), 445, 472.5, 503.5 mμ. The spectrum had a pronounced fine-structure and was indistinguishable from that of rhodopin as well as that of lycopene.

When lycoxanthin was co-chromatographed with a chromatographically homogeneous sample of all-*trans* rhodopin on a column of deactivated alumina, a clear separation into two zones was obtained. This is not in agreement with the statement of Goodwin and Land [1] that rhodopin and lycoxanthin could not be separated on an alumina column. Rhodopin was eluted with petroleum ether containing 14 % of acetone, while lycoxanthin required petroleum ether containing 25 % of acetone for elution.

Paper chromatographical studies according to the method described by Jensen *et al.*[7] gave R_F-values as presented in Table 1.

Table 1. R_F-values on Schleicher and Schüll No. 287 paper.

Carotenoid	R_F-value 5 % acetone *	10 % acetone *
Rhodopin	0.39	0.75
Lycoxanthin	0.35	0.69

* In petroleum ether bp. 60—70°C.

Again rhodopin and lycoxanthin were clearly separated into two zones. As expected, rhodopin with a shielded, tertiary hydroxyl group moved somewhat quicker than lycoxanthin with a secondary hydroxyl group, on alumina as well as on paper.

Further the IR-spectrum (KBr) of lycoxanthin was different from that of rhodopin in the 1 200—1 000 cm^{-1} region. Lycoxanthin showed a fairly strong band at 1 008 cm^{-1}. This band was absent from rhodopin, which exhibits a band of somewhat weaker intensity at 1 140 cm^{-1} characteristic of tertiary hydroxyl groups [6]. It has previously been pointed out [6] that bicyclic carotenoids with secondary hydroxyl groups all exhibit an absorption band around 1 030 cm^{-1}. The shift to 1 008 cm^{-1} for lycoxanthin is possibly due to the aliphatic nature of this carotenoid.

When treated with acetic anhydride in pyridine, lycoxanthin was quantitatively acetylated in 6 h at room temperature. Rhodopin gave no acetate at all under similar conditions. The result of the acetylation again demonstrates the different character of the hydroxyl groups of lycoxanthin and rhodopin.

* Number I, II, III of this series in *Acta Chem. Scand.* **12** (1958) 1698, **13** (1959) 381, **13** (1959) 842.

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.

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

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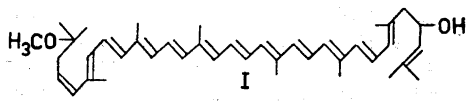
During their investigations on the carotenoids of the purple bacteria, Karrer *et al.*[1-3] described a polyene alcohol with absorption maxima in carbon disulphide at 517 and 556 mμ. In the chromatographic purification procedure employed by these workers, this carotenoid could be separated only with 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,