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# (3*R*,3′*R*)-ASTAXANTHIN FROM THE YEAST *PHAFFIA RHODOZYMA*

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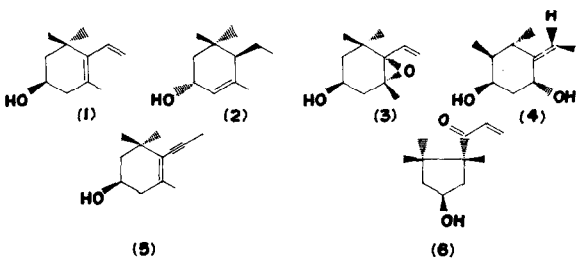
**Key Word Index**—*Phaffia rhodozyma*; Deuteromycetes; yeasts; carotenoids; astaxanthin; stereochemistry.

**Abstract**—Astaxanthin isolated from the yeast *Phaffia rhodozyma* has the 3*R*,3′*R*-configuration, opposite to that of astaxanthin from other sources which have been so far investigated. This is the first example of a naturally occurring carotenoid biosynthesized in different optical forms. A possible explanation is advanced.

## INTRODUCTION

Stereochemical investigations on carotenoids have interested a number of investigators. One of the generalizations which has arisen from the accumulated information on the absolute stereochemistry of individual carotenoids is that it is not source-dependent and that an individual carotenoid which contains one or more chiral centers always exhibits identical optical properties regardless of the source from which it is isolated. Thus, the end groups characteristic of zeaxanthin (**1**), lutein (**2**), lutein epoxide (**3**), neoxanthin (**4**), alloxanthin (**5**) and capsorubin (**6**), without exception, are always found as the same optical isomers regardless of the source of the carotenoid [1–11].




Recently [12], the absolute configuration of (3*S*,3′*S*)-astaxanthin (**7**) from *Haematococcus pluvialis* and *Homarus gammarus* was elucidated from a study of the chiroptical properties and configurational analysis of the tetrol (**8**) obtained after lithium hydride reduction of astaxanthin (**7**). Astaxanthin from *Halocynthia papillosa* [12] and an unspecified lobster [13] is also optically active and also has the same 3*S*,3′*S*-chirality. We report here the absolute configuration of astaxanthin (**9**) isolated from the red-pigmented fermenting yeast, *Phaffia rhodozyma* [14].

## RESULTS AND DISCUSSION

The isolation and identification of all-*trans* astaxanthin (**9**) from *Phaffia rhodozyma* has been described earlier [14] and the samples used in this study were from that investigation. Its CD-spectrum and that of its corresponding tetrol (**10**) obtained after $LiAlH_4$ reduction are shown in Fig. 1. For comparative purposes, the CD-spectra [12] of (3*S*,3′*S*)-astaxanthin (**7**) from *Haematococcus pluvialis* and its corresponding tetrol (**8**) are also reproduced in the same figure. The CD-curves of the two different samples of astaxanthin (**7** and **9**) are nearly identical in shape and appearance, but are directly opposite in sign. The corresponding tetrols (**8** and **10**) are also opposite to each other in sign. Astaxanthin isolated from *P. rhodozyma* therefore has the 3*R*,3′*R*-configuration. The diacetate derivative of **9** gave virtually the same CD-spectrum as the parent carotenoid.

The stereochemistry assigned to astaxanthin (**9**) isolated from *P. rhodozyma* was further supported by PMR studies using the chiral shift reagent tris[(3-heptafluorobutyryl)-*d*-camphorato]europium(III), *d*-Eu(HFC)$_3$. Identical aliquots of *d*-Eu(HFC)$_3$, after addition to equal molar solutions of 3*R*,3′*R*- and authentic 3*S*,3′*S*-astaxanthin, resulted in different lanthanide-induced shifts for the two enantiomers. At low molar ratios (0.32) of *d*-Eu(HFC)$_3$/substrate, the sense of nonequivalence was clearly reflected by the magnitude of the downfield shift observed for the double doublet assigned to the 3,3′-protons. At higher molar ratios (0.96), the 5,5′- and *gem* methyl groupings are also affected differently. In all cases the 3*S*,3′*S*-astaxanthin (**7**) exhibited larger lanthanide-induced shifts. There can be no doubt that astaxanthin isolated from *P. rhodozyma* has the 3*R*,3′*R*-configuration.

How can we explain this isolated instance of optical isomerization in carotenoids? One possible explanation may be made by considering in a stepwise manner what is known about the biosynthesis of chiral carotenoids, where the chiral center is induced by a hydroxy substituent. There is a fair body of evidence that the oxygenation reactions on carotenoids are late biosynthetic events [15–17]. The end groups characteristic of zeaxanthin (**1**) and lutein (**2**) are formed by hydroxylation reactions on $\beta$- and $\varepsilon$-rings. The observed facts are that the enzyme systems responsible for the hydroxylation reactions have identical stereochemical capacities, regardless of the organisms which effect the transformations. This property is probably related to the structure of the immediate precursor of the chiral carotenoid. Hydroxylation of an unsubstituted $\beta$-ring at C-3 always leads to the 3*R*-configuration and the same reaction on an unsubstituted $\varepsilon$-ring also yields the 3*R*-chiral end. Numerous

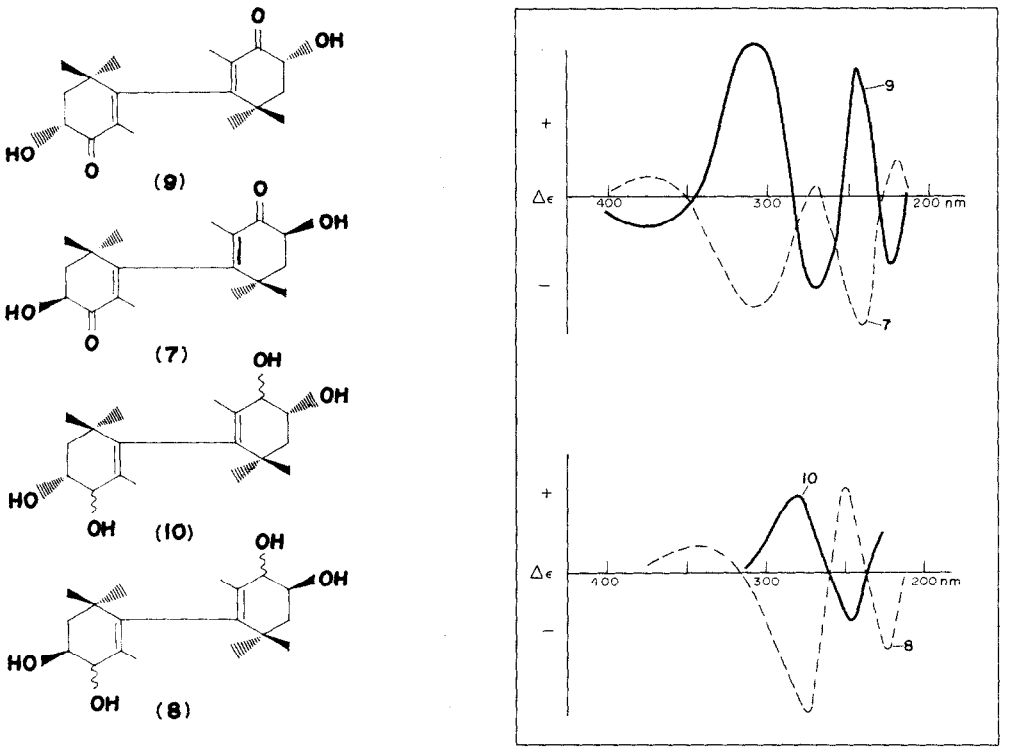
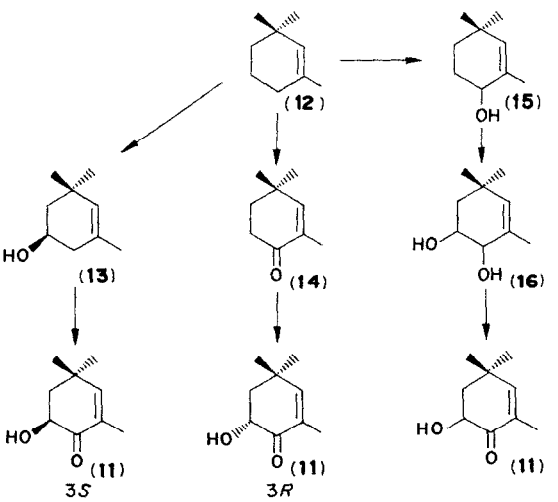



Fig. 1. CD-spectra of (3*R*,3′*R*)-astaxanthin (**9**) (top curve, solid) *ex Phaffia rhodozyma* and corresponding tetrol (**10**) (bottom curve, solid) and of (3*S*,3′*S*)-astaxanthin (**7**) (top curve, dashed) *ex Haematococcus pluvialis* and corresponding tetrol (**8**) (bottom curve, dashed).

other examples are available, including chiral centers not dependent on oxygen substituents. For example, the absolute configuration at C-6 in $C_{40}$ carotenoids with ε-ring(s) always has the same configuration [1,4,18–21]; in $C_{50}$ carotenoids, the center of asymmetry at C-2 has the same chirality [22–24].

The biosynthesis of astaxanthin differs from that of other chiral carotenoids in one important respect. The β-rings are disubstituted at position C-3 and C-4 and, because of this disubstitution, a situation arises which may account for the observed optical isomers of astaxanthin. The end group in astaxanthin (**11**) can arise [25] by three different sequential reactions (Scheme 1). The first possibility is hydroxylation of a β-end group (**12**) at C-3, followed by introduction of a keto-function at C-4, to give successively end groups **13** and **11**. One alternative mode of arriving at the astaxanthin end group (**11**) is for the keto-function at C-4 to be inserted first (**14**) followed by hydroxylation at C-3 (**11**), as shown in Scheme 1. This sequence of reactions leading to the astaxanthin end group was proposed earlier [14] for *P. rhodozyma* based on the absence in that yeast of any carotenoids with end groups **13** and **15** and the characterization of several pigments with end group **14**. The third possible pathway (Scheme 1) to the astaxanthin end group (**11**) is successive hydroxylations at C-4 (**15**) and at C-3(**16**) followed by oxidation of the C-4 hydroxyl group to a keto-group [25]. Because the precursors of the chiral end group of astaxanthin (**11**) are different, there is no reason to believe that the enzymes capable of transforming **12**–**13**, **14**–**11** and **15**–**16** would have the same stereochemical capability. It is as likely as not that one would find astaxanthin as discreet optical isomers, if these different biosynthetic routes were operating in different organisms.




Scheme 1. Three different possible biosynthetic pathways from the β-end group (**12**) to the astaxanthin end group (**11**).

From the arguments presented above, one would expect astaxanthin arising from end group **13** always to have the 3*S*,3′*S*-stereochemistry and astaxanthin from end group **14** to have the 3*R*,3′*R*-configuration. One can-

not predict the resulting sterochemistry of astaxanthin formed via **12**, **15** and **16**.

It should be interesting to measure quantitatively the chiroptical properties of astaxanthin from a variety of sources, including those where the pigment is totally synthesized, as well as those cases in which organisms are reported [25] to modify ingested dietary precursor carotenoids and convert them to astaxanthin.

## EXPERIMENTAL

The isolation of astaxanthin from the red-pigmented fermenting yeast, *Phaffia rhodozyma* strain 67-210, and its characterization has been described earlier [14]. CD-spectra were recorded in a sol of $Et_2O$–*iso*pentane–EtOH, (5:5:1) and they were confirmed independently in a different laboratory (G. Borch, personal communication). Hydride reduction of astaxanthin to the tetrol was reported earlier [12]. PMR (99.5 MHz) spectra were measured in $CDCl_3$ sol.

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