Phytochemistry, 1973, Vol. 12, pp. 2953 to 2956. Pergamon Press. Printed in England.

ISOLATION AND IDENTIFICATION OF 2-HYDROXYPLECTANIAXANTHIN FROM *RHODOTORULA AURANTIACA*

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(*Revised Received* 8 *May* 1973. *Accepted* 1 *June* 1973)

Key Word Index—*Rhodotorula aurantiaca*; cryptococcaceae; yeast; carotenoid; 2-hydroxyplectaniaxanthin

Abstract—A new trihydroxyl carotenoid has been isolated from the yeast *Rhodotorula aurantiaca* (Saito) Lodder C.B.S. 317 and identified as 2-hydroxyplectaniaxanthin (3′,4′-didehydro,1′,2′-dihydro-β, ψ-caroten-2,1′,2′-triol). Its m.p., partition coefficient, R_f, extinction coefficient, ms and NMR spectra are reported. Since the hydroxyl group at C-2 of the β-ionone ring is unusual, a possible mechanism for the biosynthesis of this carotenoid has been proposed.

INTRODUCTION

THE MAJOR carotenoids in the yeast *Rhodotorula* are β-carotene, γ-carotene, torulene and torularhodin.[1–6] However, the acidic pigment, torularhodin, is found in several but not all of the red species of *Rhodotorula*. In an extensive investigation of the carotenoids in 8 species of *Rhodotorula*, Peterson *et al.* were unable to find any torularhodin in *R. aurantiaca*, *R. pallida*, and *R. flava*, but found it in *R. mucilaginosa*, *R. glutinis* (var. *rubescens*), *R. minuta*, and *R. glutinis* in amounts ranging from 28·6 to 66·8% of the total pigment content.[3] They also reported that more polar carotenoids were included in the 84·8% of the pigments listed as torulene in *R. aurantiaca*. Recently, Bonaly and Malenge have isolated 3′,4′-didehydro-β, ψ-caroten-16′-ol, 3′,4′-didehydro-β, ψ-caroten-16′-al from *R. mucilaginosa* and *R. aurantiaca*,[7] and Bae *et al.* have identified plectaniaxanthin from *Cryptococcus laurentii*.[8]

The present paper reports the isolation of a new trihydroxyl carotenoid and plectaniaxanthin from *R. aurantiaca* (Saito) Lodder C.B.S. 317. From the absorption spectra, chemical reactions, MS and NMR spectra, the trihydroxyl carotenoid was identified as 2-hydroxyplectaniaxanthin.

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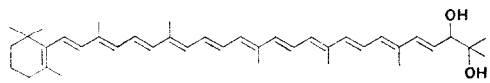
RESULTS AND DISCUSSION

The pigment extract was chromatographed on a Microcel C column and the less polar carotenoids were eluted with 3% acetone in petrol. while the dihydroxyl and trihydroxyl carotenoids were retained at the top of the column. The latter were separated by increasing the acetone content up to 10%. Usually the *cis* dihydroxyl carotenoid appeared lower on the column than the *trans* isomer, while the *cis* trihydroxyl carotenoid was above its *trans* isomer. From the visible spectra, chemical reactions (oxidation, acetylation and silylation) and the NMR spectra, the dihydroxyl carotenoid was identified as plectaniaxanthin (pigment I) which was isolated by Arpin and Jensen from the fungus *Plectania coccinea*[9] and Bae *et al.* from the yeast *Cryptococcus laurentii*.[8] Some physical data of plectaniaxanthin and the trihydroxyl carotenoid (pigment II) are shown in Table 1. Their spectra in light petrol., acetone, chloroform and benzene are identical. Pigment II melts at 175–177°. The partition coefficient (1:4–petroleum–85% MeOH) indicated that it is much more polar than plectaniaxanthin.

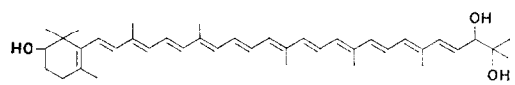
TABLE 1. CHARACTERISTICS OF PIGMENT II AND PLECTANIAXANTHIN

Carotenoids	Absorption maxima* Petrol.	Acetone	Chloroform	Benzene	Melting point	Partition coeff./P.E. 85% methanol	$E^{1\%}_{1\,cm}$ (acetone)
Pigment II	445	450	458	460	175–177°	20:80	2445
	471	476	486	488			
	502	507	519	521			
Plectaniaxanthin					169–171°	87:13	2505

When II was oxidized by *p*-chloranil and iodine in benzene,[10] its absorption maximum shifted from 471 to 494 nm. This 23 nm shift indicated the presence of one allylic hydroxyl group which was oxidized to a conjugated keto group and which should be located at the C-2′ of the acyclic end.[11] The absorption spectrum of the oxidized product was the same as that of authentic 2′-dehydroplectaniaxanthin (1′-hydroxy-3′,4′-didehydro-1′,2′-dihydro-β, ψ-caroten-2′-one). After oxidation, the R_f was increased from 0·07 to that of plectaniaxanthin (0·20) indicating the presence of two more non-allylic hydroxyl groups. When the



(I) Plectaniaxanthin



(II) 2-Hydroxyplectaniaxanthin

oxidized product was acetylated,[12] its R_f was increased from 0·20 to 0·43. The acetylated product can be further silylated[9] with a subsequent increase in R_f from 0·43 to 0·83 (Table 2). These results showed the presence of a second hydroxyl group in addition to a tertiary hydroxyl group.

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[12] LIAAEN-JENSEN, S., HEGGE, E. and JACKMAN, L. M. (1964) *Acta Chem. Scand.* **18**, 1703.
[13] ENZELL, C. R. (1969) in *Carotenoids Other Than Vitamin A*-2, p. 508, Butterworths, London.

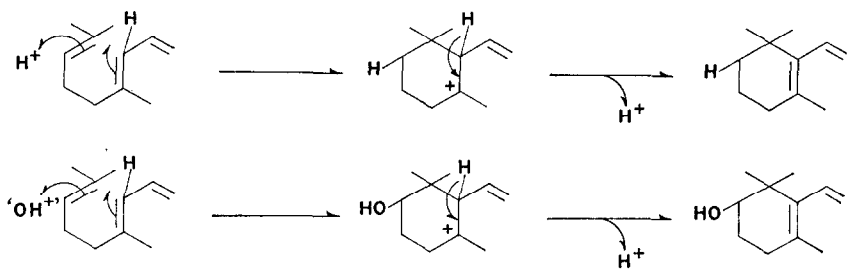
The MS of II showed a parent ion, M^+ at *m/e* 584 equivalent to that of plectanixanthin plus one oxygen atom. The fragment ions at *m/e* 568 (M-16), 566 (M-18), 550 (M-16-18), 526 (M-58), 525 (M-59), 524 (M-60), 494 (M-90), 420 (M-106-58) and 419 (M-59-106) confirm the hydroxylated acylic end group as in plectaniaxanthin.[8,13] The MS does not contain the typical fragment for the ϵ-ring and the light absorption spectrum was identical to that of plectaniaxanthin and shows the β nature of the cyclic end. It is not possible to assign the position of the third hydroxyl group from the MS except that it is on the cyclic ring. A very small peak at *m/e* 599 may indicate trace contamination of another compound.

TABLE 2. R_f OF PIGMENT II, PLECTANIAXANTHIN, 2-DEHYDROPLECTANIAXANTHIN AND THEIR CHEMICAL REACTION PRODUCTS*

Carotenoids	Original	Oxidized	Acetyl-oxidized	Silyl-acetyl-oxidized
Pigment II	0·07	0·20	0·43	0·83
Plectaniaxanthin	0·20	0·44	0·44	0·85
2′-Dehydroplectaniaxanthin	0·45	0·45	0·45	0·89

* Pigments were spotted on the silica gel sheet (Eastman chromatogram sheet 6060) 2·5 cm above the bottom. 3% MeOH in C_6H_6 was used as the mobile liquid phase. The chromatography was developed until the solvent had moved 15 cm.

The acetylation and oxidation results showed that the third hydroxyl group is neither allylic nor tertiary; thus the hydroxyl group could be located at C-2, C-3 or on the methyl groups of C-16, C-17 and C-18. The NMR spectra strongly support that this hydroxyl group is at C-2 since the methyl groups of C-1 was further split into a doublet signals at τ values of 8·90 and 8·96 (Table 3). If the hydroxyl group were on C-3, it would show a single peak for these dimethyl protons.[14] A comparison of its NMR spectrum with those of plectaniaxanthin and 2′-dehydroplectaniaxanthin shows that the splitting of the geminal dimethyl groups on C-1 and C-1′ may be due to those hydroxyl groups of C-2 and C-2′. If these hydroxyl groups were substituted with keto or acetyl groups, then no splitting could take place (Table 3). The data show that II is 2-hydroxyplectaniaxanthin (3′,4′-didehydro-1′,2′-dihydro-β, ψ-caroten-2,1′,2′-triol).



SCHEME 1. POSTULATED RING CLOSURE MECHANISMS.

A hydroxyl group on the C-2 of a β-ionone ring is unusual and may suggest a different mechanism for ring closure. Whereas a H^+ initiated ring closure has been postulated, the

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C-2 hydroxyl group might be explained by an OH^+ initiated ring closure (Scheme 1). The C-2 hydroxyl group of 2-hydroxyplectaniaxanthin may be incorporated at an early biosynthetic stage since a large amount of non-tertiary, non-allylic hydroxyphytoene and hydroxyphytofluene have been isolated from this yeast without the addition of diphenylamine.[15] The exact position of the hydroxyl groups of these compounds have not yet been determined. The consequence of different mechanisms for the ring closure of plectaniaxanthin and 2-hydroxyplectaniaxanthin would be the elimination of a precursor-product biosynthetic relationship.

TABLE 3. PMR SIGNALS OF PLECTANIAXANTHIN, 2′-DEHYDROPLECTANIAXANTHIN, PIGMENT II AND ACETYLATED PIGMENT II

Carotenoids	C-1	C-1′	C-5	C-5′	C-9,9′ C-13,13′	Acetyl group
Plectaniaxanthin	8·96	8·82 8·76	8·28	8·08	8·03	—
2′-Dehydroplectaniaxanthin	8·96	8·58	8·28	8·03	8·03	—
Pigment II	8·96 8·90	8·82 8·76	8·28	8·08	8·03	—
Acetylated pigment II	8·97	8·78 8·75: impurity	8·28	8·08	8·03	7·89 7·83

EXPERIMENTAL

Cultural condition. The yeast *Rhodotorula aurantiaca* (Saito) Lodder C.B.S. 317, kindly provided by Dr. H. J. Phaff, Department of Food Science and Technology, University of California, Davis, U.S.A., was maintained on an agar slope medium containing 5% yeast extract and 2·5% agar. 4-Day-old shake cultures (300 ml–5% glucose–0·5% yeast extract in 1 l. flask) were used to inoculate 10 l. of medium in a 14 l. microferm laboratory fermentor. The yeast was cultured for about 10 days at 23° with air being supplied at the rate of 2000 cm^3/min.

Extraction and purification of carotenoids. The yeast cells were harvested by centrifuging at 5000 rpm at 4° for 10 min. The pigments were extracted according to the procedures reported earlier.[16] The mixture was chromatographed on Microcel C columns and eluted with 3% acetone in light petrol. until the less polar carotenoids were eluted from the column while the more polar compounds could be separated into bands in the order of *cis* plectaniaxanthin, *trans* plectaniaxanthin, *trans* 2-hydroxyplectaniaxanthin and *cis* 2-hydroxyplectaniaxanthin by increasing the acetone content gradually to 10%. The column material was extruded, the pigments removed by acetone and transferred into light petrol. by washing with H_2O. Each carotenoid was rechromatographed on a Microcel C column and eluted with 5–10% acetone in light petrol. 2-Hydroxyplectaniaxanthin and plectaniaxanthin were precipitated during concentration of the light petrol. extract, collected and washed.

NMR. The NMR spectra were determined at the Chemistry Department, University of Rhode Island, Kingston, Rhode Island, U.S.A. and U.S.D.A. Fruit and Vegetable Chemistry Laboratory, Pasadena, California, U.S.A. The frequencies used for the NMR spectra of pigment II, acetylated pigment II and 2′-dehydro plectaniaxanthin were 60 MHz and plectaniaxanthin was 100 MHz. They were all measured in $CDCl_3$ with TMS as reference. The positions of the signals were given in τ values.

MS. The MS were determined by Dr. G. Britton, Department of Biochemistry, University of Liverpool. The spectrum of II was measured at the ionization voltage of 70 eV and the temp. of the ion chamber was kept at 200°.

Acknowledgements—The authors would like to thank Dr. G. Britton and Mr. D. Dauplaise for the measurements of MS and NMR spectra, respectively. The work was supported by grant AM-11665 from U.S.P.H.S. to Dr. C. O. Chichester. This paper is contribution number 1444 of the University of Rhode Island Agricultural Experiment Station, Kingston, Rhode Island, U.S.A.

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